Procleix® Ultrio® Assay

For *In Vitro* Diagnostic Use
1000 Test Kit, 5000 Test Kit

CONTENTS

GENERAL INFORMATION

INTENDED USE

The PROCLEIX® ULTRIO® Assay* is a qualitative in vitro nucleic acid assay system to screen for human immunodeficiency virus type I (HIV-1) RNA and hepatitis C virus (HCV) RNA in plasma and serum specimens from individual human donors, including donors of whole blood and blood components, source plasma and other living donors. It is also intended for use in testing plasma and serum specimens to screen organ donors when specimens are obtained while the donor's heart is still beating, and in testing blood specimens from cadaveric (non-heart-beating) donors. The assay is not intended for use on cord blood specimens.

The assay is intended for use in testing individual samples from living donors of whole blood, blood components, or source plasma, other living donors and heart-beating organ donors, and for testing individual blood specimens from cadaveric (non-heart-beating) donors. It is also intended for use in testing pools of human plasma comprised of equal aliquots of not more than 16 individual donations from donors of whole blood, blood components, or source plasma. This assay is intended to be used in conjunction with licensed tests for detecting antibodies to HIV-1 and HCV.

The PROCLEIX ULTRIO Assay is not intended for use to screen donor specimens for HBV DNA. The assay detects HBV DNA in HBV seroconversion panel specimens that are negative for hepatitis B surface antigen (HBsAg) and antibodies to hepatitis B core antigen (anti-HBc). The assay also detects HBV DNA in donor specimens that are positive for HBsAg and/or anti-HBc. However, detection of HBV DNA in donations negative for both HBsAg and anti-HBc has not been demonstrated in the donor setting.

This assay is not intended for use as an aid in diagnosis of infection with HIV-1, HCV or HBV.

SUMMARY AND EXPLANATION OF THE TEST

Epidemiological studies identified human immunodeficiency virus type 1 (HIV-1) as the etiological agent of acquired immunodeficiency syndrome (AIDS)[1–7] hepatitis C virus (HCV)[8–13] as the etiological agent for most blood-borne non-A, non-B hepatitis (NANBH), and hepatitis B virus (HBV) as the etiological agent for infectious serum hepatitis. HIV-1, HCV, and HBV are transmitted primarily by exposure to infected blood or blood products, certain body fluids or tissues, and from mother to fetus or child.

Current detection of HIV-1 infection in the blood bank setting is based on Nucleic Acid Testing (NAT) for HIV-1 RNA detection[27, 28, 30, 31] and/or serologic screening for anti-viral antibodies, with confirmation by supplemental antibody tests such as Western blot or immunofluorescence assays. In addition, depending on the NAT assay of use, p24Ag assays followed by confirmation by neutralization are used. The recent addition of nucleic acid-based amplification tests has reduced the window period of detection by 6 to 11 days, preventing more than half of the HIV-1 infections by blood transfusion.[15, 16, 17, 29]

Current detection of HCV infection in the blood bank setting is based on NAT for HCV RNA detection[27, 28, 30, 31] and/or serologic screening for anti-viral antibodies and confirmation with a Strip Immunoblot Assay (e.g., CHIRON® RIBA® HCV 3.0 SIA). The recent introduction of nucleic acid-based amplification tests for HCV RNA has allowed detection of HCV infection approximately 59 days earlier than the current antibody-based tests.[17, 29]

Current detection of HBV infection in the blood bank setting is based on serological screening for HBsAg, with confirmation by confirmatory neutralization tests, and for anti-HBc. Data from post-transfusion cases indicate that HBsAg is first detected 50 to 60 days following transfusion.[14]

The PROCLEIX® ULTRIO® Assay utilizes target amplification nucleic acid probe technology for the detection of HIV-1 RNA, HCV RNA, and HBV DNA.[18, 27] The assay contains reagents which may be used for simultaneous detection of all three viruses or the individual viruses: HIV-1, HCV, and HBV. The PROCLEIX® Assays incorporate an Internal Control for monitoring assay performance in each individual specimen.

PRINCIPLES OF THE PROCEDURE

The PROCLEIX® ULTRIO® Assay involves three main steps, which take place in a single tube: Sample Preparation; HIV-1 RNA, HCV RNA and HBV DNA target amplification by Transcription-Mediated Amplification (TMA)[19]; and detection of the amplification products (amplicon) by the Hybridization Protection Assay (HPA).[20]

During Sample Preparation, viral RNA and DNA are isolated from specimens via the use of target capture. The specimen is treated with a detergent to solubilize the viral envelope, denature proteins and release viral genomic RNA and/or DNA. Oligonucleotides (capture oligonucleotides) that are homologous to highly conserved regions of HIV-1, HCV, and HBV are hybridized to the HIV-1 RNA, HCV RNA, or HBV DNA target, if present, in the test specimen. The hybridized target is then captured onto magnetic microparticles that are separated from the specimen in a magnetic field. Wash steps are utilized to remove extraneous components from the reaction tube. Magnetic separation and wash steps are performed with a target capture system.

Target amplification occurs via TMA, which is a transcription-based nucleic acid amplification method that utilizes two enzymes, MMLV reverse transcriptase and T7 RNA polymerase. The reverse transcriptase is used to generate a DNA copy (containing a promoter sequence for T7 RNA polymerase) of the target sequence. T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template. The PROCLEIX ULTRIO Assay utilizes the TMA method to amplify regions of HIV-1 RNA, HCV RNA, and/or HBV DNA.

Detection is achieved by HPA using single-stranded nucleic acid probes with chemiluminescent labels that are complementary to the amplicon. The labeled nucleic acid probes hybridize specifically to the amplicon. The Selection Reagent differentiates between hybridized and unhybridized probes by inactivating the label on unhybridized probes. During the detection step, the chemiluminescent signal produced by the hybridized probe is measured in a luminometer and is reported as Relative Light Units (RLU).

Internal Control is added to each test specimen, control (if used), or assay calibrator tube via the working Target Capture Reagent that contains the Internal Control. The Internal Control in this reagent controls for specimen processing, amplification, and detection steps. Internal Control signal in each tube or assay reaction is discriminated from the HIV-1/HCV/HBV signal by the differential kinetics of light emission from probes with different labels.[21] Internal Control-specific amplicon is detected using a probe with rapid emission of light (flasher signal). Amplicon specific to HIV-1/HCV/HBV is detected using probes with relatively slower kinetics of light emission (glower signal). The Dual Kinetic Assay (DKA) is a method used to differentiate between the signals from flasher and glower labels.[21] When used for the simultaneous detection of HIV-1, HCV, and HBV, the PROCLEIX® ULTRIO® Assay differentiates between Internal Control and combined HIV-1/HCV/HBV signals but does not discriminate between individual HIV-1, HCV, and HBV signals.

Specimens found to be reactive in the PROCLEIX ULTRIO Assay must be run in individual HIV-1, HCV, and/or HBV Discriminatory Assays to determine if they are reactive for HIV-1, HCV, HBV or any combination of the three.

The PROCLEIX® HIV-1, HCV, and HBV Discriminatory Assays utilize the same three main steps as the PROCLEIX ULTRIO Assay (target capture, TMA and HPA); the same assay procedure is followed with one difference: HIV-1-specific, HCV-specific, or HBV-specific probe reagents are used in place of the PROCLEIX ULTRIO Assay Probe Reagent.

* Developed by Gen-Probe in collaboration with Chiron; Manufactured by Gen-Probe Incorporated; Distributed by Chiron Corporation

REAGENTS

PROCLEIX® ULTRIO® Assay Kit:

CONTENTS:	Number of vials/ Volume per vial	
Reagent Name	**1000 Test Kit**	**5000 Test Kit**
Internal Control Reagent *A HEPES buffered solution containing detergent and an RNA transcript.* *Store **unopened reagent** at –15° to –35°C.*	**2 x 5 mL**	**10 x 5 mL**
Target Capture Reagent *A HEPES buffered solution containing detergent, capture oligonucleotides and magnetic microparticles.* *Store at 2° to 8°C. (Do not freeze)* Internal Control Reagent must be added to Target Capture Reagent before use in the assay.	**2 x 280 mL**	**10 x 280 mL**
Amplification Reagent *Primers, dNTPs, NTPs and co-factors in TRIS buffered solution containing PROCLIN® 300 as preservative.* *Store **unopened reagent** at –15° to –35°C.*	**2 x 50 mL**	**10 x 50 mL**
Enzyme Reagent *MMLV Reverse Transcriptase and T7 RNA Polymerase in HEPES/ TRIS buffered solution containing 0.05% sodium azide as preservative.* *Store **unopened reagent** at –15° to –35°C.*	**2 x 18 mL**	**10 x 18 mL**
Probe Reagent *Chemiluminescent oligonucleotide probes in succinate buffered solution containing detergent.* *Store **unopened reagent** at –15° to –35°C.*	**2 x 75 mL**	**10 x 75 mL**
Selection Reagent *Borate buffered solution containing surfactant.* *Store at 15° to 30°C.*	**2 x 180 mL**	**10 x 180 mL**
PROCLEIX® Negative Calibrator *Defibrinated normal human plasma (nonreactive for HIV-1/2, HCV, and HBV when tested by FDA-licensed assays) containing gentamicin and 0.2% sodium azide as preservatives.* *Store at –15° to –35°C.*	**30 x 2 mL** **C0**	**90 x 2 mL**
PROCLEIX® HIV-1 Positive Calibrator *Inactivated HIV-1 positive plasma in defibrinated normal human plasma (nonreactive for HIV-2, HCV, and HBV when tested by FDA-licensed assays) containing gentamicin and 0.2% sodium azide as preservatives.* *Store at –15° to –35°C.*	**30 x 2 mL** **C1**	**90 x 2 mL**
PROCLEIX® HCV Positive Calibrator *Inactivated HCV positive plasma in defibrinated normal human plasma (nonreactive for HIV-1/2 and HBV when tested by FDA-licensed assays) containing gentamicin and 0.2% sodium azide as preservatives.* *Store at –15° to –35°C.*	**30 x 2 mL** **C2**	**90 x 2 mL**
PROCLEIX® HBV Positive Calibrator *Inactivated HBV positive plasma in defibrinated normal human plasma (nonreactive for HIV-1/2 and HCV when tested by FDA-licensed assays) containing gentamicin and 0.2% sodium azide as preservatives.* *Store at –15° to –35°C.*	**30 x 2 mL** **C3**	**90 x 2 mL**

STORAGE INSTRUCTIONS

A. Room temperature is defined as 15° to 30°C.

B. ⚠ The PROCLEIX® ULTRIO® Assay Probe Reagent and the Discriminatory Probe Reagents are light-sensitive. Protect these reagents from light during storage and preparation for use.

C. Do not use reagents or fluids after the expiration date.

D. Do not use assay-specific reagents from any other PROCLEIX® Assay.

E. If a precipitate forms in the Target Capture Reagent (TCR) during storage, see instructions under REAGENT PREPARATION. DO NOT VORTEX. DO NOT FREEZE TCR.

Note: If after removing the TCR from storage at 2° to 8°C, the precipitate is allowed to settle to the bottom of the container, the likelihood of the formation of a gelatinous precipitate is increased substantially.

F. Do not refreeze Internal Control, Amplification, Enzyme, Probe, HIV-1, HCV, and HBV Discriminatory Probe Reagents after the initial thaw.

G. Calibrators are single use vials and must be discarded after use.

H. If precipitate forms in the Wash Solution, Amplification Reagent, Selection Reagent, Probe Reagent, or HIV-1, HCV, or HBV Discriminatory Probe Reagents, see instructions under REAGENT PREPARATION.

I. Changes in the physical appearance of the reagent supplied may indicate instability or deterioration of these materials. If changes in the physical appearance of the reagents are observed once resuspended (e.g., obvious changes in reagent color or cloudiness indicative of microbial contamination), they should not be used.

J. Consult the following table for storage information.

Reagent/Fluid	Unopened Storage	Opened/Thawed Stability (up to expiration date)
Internal Control Reagent (IC)	-15° to -35°C until the expiration date	Prior to combining with TCR, 8 hours at RT*
Target Capture Reagent (TCR)	2° to 8°C until the expiration date	
working Target Capture Reagent (wTCR)		30 days at 2° to 8°C; 80 hours at RT**
Probe Reagents	-15° to -35°C until the expiration date	30 days at 2° to 8°C; 80 hours at RT**
Amplification Reagent	-15° to -35°C until the expiration date	30 days at 2° to 8°C; 80 hours at RT**
Enzyme Reagent	-15° to -35°C until the expiration date	30 days at 2° to 8°C; 80 hours at RT**
Selection Reagent	RT until the expiration date	30 days at RT
Calibrators	-15° to -35°C until the expiration date	8 hours at RT
Auto Detect Reagents	RT until the expiration date	30 days at RT
Buffer for Deactivation Fluid	RT until the expiration date	30 days at RT
Deactivation Fluid	N/A	30 days at RT
Oil	RT until the expiration date	30 days at RT
Wash Solution	RT until the expiration date	30 days at RT

* RT = Room Temperature

** The 80 hours must occur within the 30 days.

SPECIMEN COLLECTION, STORAGE, AND HANDLING

Note: Handle all specimens as if they are capable of transmitting infectious agents.

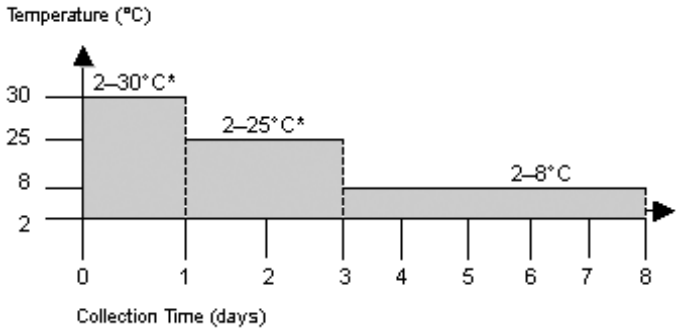
Note: Take care to avoid cross-contamination during the sample handling steps. For example, discard used material without passing over open tubes.

Living Donor Blood Specimens

A. Blood specimens collected in glass or plastic tubes may be used.

B. Plasma collected in K_2EDTA, K_3EDTA, ACD, sodium citrate, or in Becton-Dickinson EDTA Plasma Preparation Tubes (BD PPT™) may be used. Follow sample tube manufacturer's instructions. Specimen stability is affected by elevated temperature. Whole blood or plasma from pooled or individual donor specimens may be stored for up to 72 hours from time of draw at ≤ 25°C; temperatures not to exceed 30°C are acceptable for no more than 24 hours, including time on instrument. Specimens may be stored an additional five days at 2° to 8°C following centrifugation. Plasma separated from the cells may be stored for up to 6 months at ≤ -20°C before testing. Do not freeze whole blood.

C. Additional specimens collected in serum tubes or heparin tubes according to the collection container manufacturer's instructions, may be used. Whole blood, plasma, or serum may be stored for up to 72 hours from time of draw at ≤ 25°C; temperatures not to exceed 30°C are acceptable for no more than 24 hours, including time on instrument. Specimens may be stored an additional five days at 2° to 8°C following centrifugation. Long-term storage of serum and heparinized plasma has not been evaluated.



***The 2-30° and 2-25°C periods indicated above may occur at any time.**

D. Additional specimens may be taken from whole blood or plasma units containing CPD, CP2D, or CPDA-1 anticoagulants collected according to the collection container manufacturer's instructions. Whole blood (not plasma units) collected in these anticoagulants may be stored for up to 13 days at 2° to 8°C prior to centrifugation. At any time within this 13-day period, the whole blood unit or aliquot for testing may have been stored for up to one day at 30°C and up to two days at 25°C, including time on instrument. Following centrifugation, the plasma may be stored for an additional five days at 2° to 8°C before testing. Plasma separated from the cells may be stored for up to 6 months at ≤ -20°C before testing.

E. No adverse effect on assay performance was observed when plasma or serum was subjected to three freeze-thaw cycles.

F. Specimens with visible precipitates or fibrinous material should be clarified by centrifugation for 10 minutes at 1000 to 3000 x g prior to testing. Do not test specimens that do not have sufficient sample volume above the gel separator or red cell interface.

G. Mix thawed plasma or serum thoroughly and centrifuge for 10 minutes at 1000 to 3000 x g before testing. Centrifugation times and speeds for thawed BD PPT tubes must be validated by the user.

H. If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents.[25]

I. False positive results may occur if cross-contamination of specimens is not adequately controlled during specimen handling and processing.

J. Specimen Pooling

The pooling software, used in combination with a front end pipettor, performs sample scanning and pooling operations that combine aliquots from individual samples into a single Master Pool Tube, which may be used for further testing. See OTHER MATERIALS AVAILABLE FROM CHIRON FOR USE WITH PROCLEIX® ULTRIO® ASSAY for a listing of specific pooling materials.

***Note:* Only specimens from donors of whole blood, blood components, or source plasma may be pooled.**

Cadaveric Blood Specimens

Note: **A serum or plasma specimen collected pre-mortem from a non-heart-beating (cadaveric) organ/cell/tissue donor may be tested instead of a cadaveric blood specimen using instructions for cadaveric donors.**

A. Cadaveric blood specimens can be collected in clot or EDTA anti-coagulant tubes. Follow sample tube manufacturer's instructions.

B. For collection of specimens from cadaveric donors, follow general standards and/or regulations. Specimen stability is affected by elevated temperature.

C. Whole blood (EDTA collection tube) or plasma may be stored for up to 72 hours at 2° to 8°C; temperatures not to exceed 25°C are acceptable for no more than 24 hours. Specimens may be stored an additional 5 days at 2° to 8°C following centrifugation. Long-term storage for plasma at ≤ -20°C has not been established. Do not freeze whole blood.

D. Whole blood (clot tube) or serum may be stored for up to 72 hours at 2° to 8°C; temperatures not to exceed 25°C are acceptable for no more than 24 hours. Specimens may be stored for an additional two days at 2° to 8°C following centrifugation. Long-term storage for serum at ≤ -20°C has not been established. Do not freeze whole blood.

E. No adverse effect on assay performance was observed when plasma and serum were subjected to three freeze-thaw cycles.

F. Specimens with visible precipitates or fibrinous material should be clarified by centrifugation for 10 minutes at 1000 to 3000 x g prior to testing. Do not test specimens that do not have sufficient sample volume above the gel separator or red cell interface.

G. Mix thawed plasma or serum thoroughly and centrifuge for 10 minutes at 1000 to 3000 x g before testing. Centrifugation times and speeds for thawed BD PPT tubes must be validated by the user.

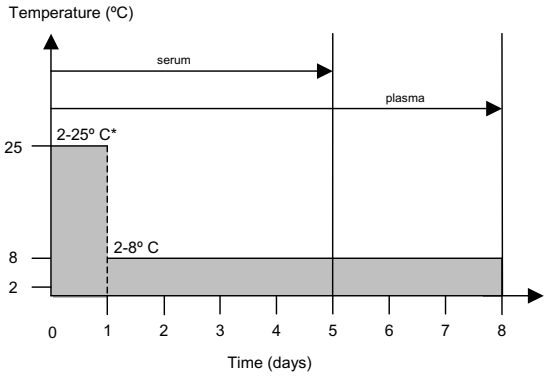
H. If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents.[25]

I. False positive results may occur if cross-contamination of specimens is not adequately controlled during specimen handling and processing.

J. Cadaveric blood specimens may be diluted to overcome potential sample inhibitory substances or specimen shortage. Plasma and/or serum may be diluted 1:5 in saline (0.9% sodium chloride), i.e. 100 µL sample plus 400 µL saline. Diluted specimens should be inverted several times to mix and then may be used in standard assay procedure by pipetting the 500 µL of the diluted specimen into the TTU containing TCR.

Note: If a front-end pipettor will be used to pipette the samples, the minimum volume for the diluted sample should be 1100 µL (220 µL neat sample plus 880 µL saline).

Note: Studies performed to validate these conditions were performed on negative cadaveric specimens spiked with virus. The stability of HIV-1, HCV, and HBV *in vivo* post-mortem was not assessed.



***The 2-25° period indicated above may occur at any time.**

PROCLEIX® SYSTEM USERS

MATERIALS PROVIDED

PROCLEIX® ULTRIO® Assay **1000 Test Kit** P/N 301102
5000 Test Kit P/N 301104

Internal Control Reagent
Target Capture Reagent
Amplification Reagent
Enzyme Reagent
Probe Reagent
Selection Reagent
PROCLEIX® Negative Calibrator
PROCLEIX® HIV-1 Positive Calibrator
PROCLEIX® HCV Positive Calibrator
PROCLEIX® HBV Positive Calibrator

MATERIALS REQUIRED BUT PROVIDED SEPARATELY

PROCLEIX® HIV-1, HCV, and HBV Discriminatory Probe Reagents

HIV-1 Discriminatory Probe Reagent	P/N 302165
HCV Discriminatory Probe Reagent	P/N 302163
HBV Discriminatory Probe Reagent	P/N 302164

PROCLEIX® Assay Fluids P/N 301116

Wash Solution
Oil
Buffer for Deactivation Fluid

PROCLEIX® Auto Detect Reagents P/N 301120

Auto Detect 1
Auto Detect 2

Disposables

(Disposables are single use only, do not reuse. Use of other disposables is not recommended.)

Ten-Tube Units (TTUs)	P/N TU0040
Ten Tip Cassettes	P/N 104578
Sealing Cards	P/N 102085

Equipment/Software

PROCLEIX® System:
TECAN GENESIS RSP instrument (front end pipettor), PROCLEIX® Assay Software, and operator's manual; or PROCLEIX® Worklist Editor software and operator's manual
PROCLEIX® TCS and operator's manual
PROCLEIX® HC+ Luminometer, PROCLEIX® ULTRIO® System Software, and operator's manual
Multi-tube Vortex Mixer (Vortexer)
Water bath
Dedicated fixed or adjustable repeat pipettors capable of delivering 25-500 µL of liquid with a ± 5% accuracy and a coefficient of variation of ≤ 5%.

Other

PROCLEIX® System Quick Reference Guide (PROCLEIX System QRG)
Any applicable technical bulletins

OTHER MATERIALS AVAILABLE FROM CHIRON FOR USE WITH PROCLEIX® ULTRIO® ASSAY

PROCLEIX® ULTRIO® Assay Calibrators P/N 302166

PROCLEIX® Negative Calibrator
PROCLEIX® HIV-1 Positive Calibrator
PROCLEIX® HCV Positive Calibrator
PROCLEIX® HBV Positive Calibrator

General Equipment/Software

PROCLEIX® CPT Pooling Software and operator's manual

For instrument specifics and ordering information, contact Chiron Customer Support.

MATERIALS REQUIRED BUT NOT PROVIDED

Repeat pipettor tips (1.25 mL, 5.0 mL, 10.0 mL, 12.5 mL)

If using the Manual Sample Pipetting Method: Filtered fixed pipettor tips capable of delivering 500 µL (for samples) and repeat pipettor tips capable of delivering 400 µL (for wTCR)

If using the TECAN GENESIS RSP instrument: Disposable conductive filter tips in rack approved for use with equipment and front end pipettor reagent troughs

Bleach

For use in final concentrations of 5% sodium hypochlorite and 0.5% sodium hypochlorite

Bleach alternative (optional)

Contact Chiron Technical Support for a list of bleach alternatives and instructions for use.

Sterile, polypropylene conical tubes with sealing caps. Freestanding tubes are recommended in two different sizes (5 mL to 10 mL tube and ≥ 30 mL tube). The tubes must be able to accommodate the diameter of a repeat pipettor tip.

PRECAUTIONS

A. For *in vitro* diagnostic use.

B. When performing testing with different PROCLEIX® Assays using shared instrumentation, ensure appropriate segregation is maintained to prevent mix-up of samples during processing (e.g., use of colored TTU racks). In addition, verify that the correct set of reagents is being used for the assay that is being run.

C. To reduce the risk of invalid results, carefully read the entire package insert for the PROCLEIX® ULTRIO® Assay and the PROCLEIX® System QRG prior to performing an assay run.

D. Specimens may be infectious. Use Universal Precautions[22, 26] when performing the assay. Proper handling and disposal methods should be established according to local, state and federal regulations.[23, 24, 25] Only personnel adequately qualified as proficient in the use of the PROCLEIX® ULTRIO® Assay and trained in handling infectious materials should perform this procedure.

E. **CAUTION: Some components of this kit contain human blood products. The HIV-1 Positive Calibrator in this kit contains human plasma that is HIV-1 positive and has been heat-treated to inactivate the virus. The HCV Positive Calibrator contains human plasma that is HCV positive and has been heat-treated to inactivate the virus. The HBV Positive Calibrator contains human plasma that is HBV positive and has been heat-treated to inactivate the virus. The Negative Calibrator has been assayed by FDA-licensed tests and found nonreactive for HIV-1/2, HCV, and HBV. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. All human blood sourced materials should be considered potentially infectious and should be handled with Universal Precautions.[22, 26] If spillage occurs, immediately disinfect, then wipe up with a 0.5% (final concentration) sodium hypochlorite solution (diluted bleach) or follow appropriate site procedures. A bleach alternative may be used in Pre-Amplification areas only. Do not use bleach alternatives in Amplification areas or in areas suspected to be contaminated with Amplification products.**

F. Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink or smoke in designated work areas. Wear disposable gloves and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

G. This product contains sodium azide as a preservative. Do not use metal tubing for reagent transfer. If solutions containing azide compounds are disposed of in a plumbing system, they should be diluted and flushed with generous amounts of running water. These precautions are recommended to avoid accumulation of deposits in metal piping in which explosive conditions could develop.

H. Avoid contact of Auto Detect Reagents 1 and 2 with skin, eyes and mucous membranes. Wash with water if contact with these reagents occurs. If spills of these reagents occur, dilute with water and follow appropriate site procedures.

I. Dispose of all materials that have come in contact with specimens and reagents according to local, state and federal regulations.[23, 24] Thoroughly clean and disinfect all work surfaces.

J. Use only supplied or specified required disposables.

K. Do not use this kit after its expiration date. DO NOT interchange, mix, or combine reagents from kits with different master lot numbers.

L. Avoid microbial and ribonuclease contamination of reagents.

M. Store all assay reagents at specified temperatures. The performance of the assay may be affected by use of improperly stored assay reagents. See STORAGE INSTRUCTIONS and REAGENT PREPARATION for specific instructions.

N. Store all specimens at specified temperatures. The performance of the assay may be affected by use of improperly stored specimens. See SPECIMEN COLLECTION, STORAGE, AND HANDLING for specific instructions.

O. Only combine assay reagents or fluids as instructed to by the PROCLEIX® ULTRIO® Assay package insert.

P. Some reagents of this kit are labeled with risk and safety symbols according to the European Directive 1999/45/EC and should be handled accordingly.

Material Safety Data Sheets are available upon request.

The following reagents contain 0.2% sodium azide as a preservative. They may also pose a potential biological risk:
PROCLEIX® Negative Calibrator
PROCLEIX® HIV-1 Positive Calibrator
PROCLEIX® HCV Positive Calibrator
PROCLEIX® HBV Positive Calibrator

Xn. Harmful

Biological Risk

R22/R32/S2/
S13/S36/S46

R22 Harmful if swallowed
R32 Contact with acid liberates very toxic gas
S2 Keep out of reach of children
S13 Keep away from food, drink, and animal feeding stuffs
S36 Wear suitable protective clothing
S46 If swallowed, seek medical advice immediately and show this container or label

Q. Refer to precautions in the appropriate PROCLEIX® Assay package inserts, operator's manuals, and the PROCLEIX System QRG.

REAGENT PREPARATION

These steps should be performed prior to beginning Target Capture in an area that is free of template and amplicon.

A. Room temperature is defined as 15° to 30°C.

B. Verify that reagents have not exceeded the expiration date and/or storage stability times.

C. Remove a bottle of Selection Reagent from room temperature storage.

1. The Selection Reagent must be at room temperature before use.
2. If Selection Reagent has been inadvertently stored at 2° to 8°C or the temperature of the laboratory falls between 2° and 15°C, precipitate may form.
3. If precipitate forms in the Selection Reagent, heat at 60° ± 1° C for no more than 45 minutes, shaking the bottle frequently (every 5 to 10 minutes). Once all precipitate has gone back into solution, place the bottle in a room temperature water bath and allow the bottle to equilibrate for at least 1 hour. Do not use the Selection Reagent until it has equilibrated.
4. If foam is present, carefully remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.
5. Do not use if precipitate or cloudiness persists.
6. Record the date that it was first opened (OPEN DATE) on the space provided on the label.

D. Warm all reagents to room temperature and mix thoroughly prior to use. A dedicated water bath at room temperature may be used to aid this process.

1. If using a water bath, thaw reagents upright.
2. Amplification, HIV-1 Discriminatory Probe, HCV Discriminatory Probe, HBV Discriminatory Probe, and Probe Reagents may be mixed by vortexing.
3. Enzyme Reagent should be mixed thoroughly by gentle inversion, taking care to avoid excessive foaming.
4. Record the date of thaw (THAW DATE) for each reagent on the space provided on the label.

E. Ensure that precipitates are dissolved. Do not use a reagent if precipitate or cloudiness is present.

F. DO NOT heat Probe Reagents above 30°C if using a water bath.

G. The PROCLEIX® ULTRIO® Assay Probe Reagent and the Discriminatory Probe Reagents are light-sensitive. Protect these reagents from light during storage and preparation for use.

H. Precipitate will form in the Probe and Discriminatory Probe Reagents when stored at 2° to 8°C. Probe Reagents may be warmed in a water bath to facilitate dissolution of precipitate, but temperature in the water bath should not exceed 30°C. If thawing is conducted on the lab bench, Probe Reagents may take up to 4 hours with periodic mixing to allow complete dissolution of precipitate. Ensure that precipitates in the Probe Reagents are dissolved. Do not use if precipitate or cloudiness is present.

I. Prepare working Target Capture Reagent (wTCR):

1. Remove TCR from 2° to 8°C storage. IMMEDIATELY upon removing from storage, mix vigorously (at least 10 inversions). DO NOT VORTEX.
2. After mixing, place the TCR bottle at 22° to 30°C. Approximately every 10 minutes shake the bottle until all precipitate has disappeared. TCR precipitate should normally dissolve in about 30 minutes.

   Note: If a gel is observed after performing this procedure, a new bottle must be used according to the handling recommendations above. Return the bottle with gel back to 2° to 8°C storage for subsequent use.
3. Thaw one vial of Internal Control Reagent up to 24 hours at 2° to 8° C or up to 8 hours at room temperature.
4. Mix the Internal Control Reagent thoroughly by inversion or vortexing.
5. When the Internal Control Reagent and TCR have reached room temperature, mix TCR thoroughly by inversion. Pour the entire vial of Internal Control Reagent into the TCR bottle. This is now the working Target Capture Reagent (wTCR). Mix thoroughly.
6. Use the space indicated on the TCR bottle to record the date Internal Control Reagent was added and lot number used (IC LOT). Record the expiration date of the wTCR in the space provided on the label.

J. Thaw calibrators at room temperature.

1. These are single use vials and must be thawed prior to each run.
2. Mix calibrators gently by inversion to avoid foaming.
3. If foam is present, carefully remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.

K. Wash Solution is shipped at ambient temperature and stored at room temperature. Precipitates may form in the Wash Solution during shipment or during storage when temperatures fall between 2° and 15°C. Wash Solution may be warmed to facilitate dissolution of precipitate. Temperature should not exceed 30°C. Ensure that precipitates in the Wash Solution are dissolved prior to use. Do not use if precipitate or cloudiness is present.

L. For Wash Solution, Oil, Selection Reagent, Buffer for Deactivation Fluid, Auto Detect 1, and Auto Detect 2, record the date the reagent was first opened (OPEN DATE) in the space provided on the label.

M. To prepare Deactivation Fluid, mix one part Buffer for Deactivation Fluid with one part 5% sodium hypochlorite. Record the date the Deactivation Fluid was prepared.

PROCEDURAL NOTES

Note: Refer to the PROCLEIX® System QRG for maintenance procedures and information about software operation.

A. To reduce the risk of invalid results, carefully read the entire package insert for the PROCLEIX® ULTRIO® Assay prior to performing an assay run. This package insert must be used with the PROCLEIX® System QRG and any applicable technical bulletins.

B. RUN SIZE

1. Kit test size is based on an average run size of 55 tests. Smaller run sizes will result in a lower yield.
2. Each PROCLEIX® ULTRIO® Assay run will yield up to 100 test results, including results for external quality controls (if used), three replicates of the Negative Calibrator, and two replicates each of the HIV-1 Positive Calibrator, the HCV Positive Calibrator, and the HBV Positive Calibrator.
3. Each PROCLEIX® HIV-1, HCV, or HBV Discriminatory Assay run will yield up to 100 test results, including results for external quality controls (if used), three replicates of the Negative Calibrator, three replicates of the corresponding Positive Calibrator, and two replicates of each of the other two Positive Calibrators.

C. EQUIPMENT PREPARATION

1. Three dedicated water baths must be used: one for target capture and pre-amplification (60° ± 1°C), one for amplification (41.5° ± 1°C) and one for hybridization and selection (62° ± 1°C). An additional container of water is required to be maintained at 23° ± 4°C for the step preceding detection.
2. Equilibrate water baths to 60° ± 1°C for target capture and 41.5° ± 1°C for amplification incubations.
3. If using a front end pipettor, set up according to instructions in the PROCLEIX System QRG.
4. Prepare the target capture system according to instructions in the PROCLEIX System QRG.
5. Wipe work surfaces and pipettors daily with diluted bleach (0.5% sodium hypochlorite in water). Allow bleach to contact surfaces and pipettors for at least 15 minutes and then follow with a water rinse. A bleach alternative may be used in Pre-Amplification areas only. **Do not use bleach alternatives in Amplification areas or in areas suspected to be contaminated with Amplification products.** DO NOT USE DEACTIVATION FLUID ON SURFACES.
6. Equilibrate a water bath to 62° ± 1°C for hybridization and selection incubations. Equilibrate a container of water at 23° ± 4°C for cool down prior to detection.
7. Prepare the luminometer according to instructions in the PROCLEIX System QRG.

D. REAGENTS

1. Add all reagents using a repeat pipettor capable of delivering specified volume with ± 5% accuracy and a precision of ≤ 5% CV. Check pipettor functionality monthly and calibrate regularly.
2. To minimize waste of Amplification, Oil, Enzyme, HIV-1 Discriminatory Probe, HCV Discriminatory Probe, HBV Discriminatory Probe, Probe, and Selection Reagents, aliquot each reagent for a given run size. Aliquoting must be performed after reagent preparation using sterile, polypropylene conical tubes with sealing caps in an area that is template and amplicon free. The aliquoting area must be wiped down with diluted bleach (0.5% sodium hypochlorite in water) before and after the aliquoting process. A bleach alternative may be used in Pre-Amplification areas only. **Do not use bleach alternatives in Amplification areas or in areas suspected to be contaminated with Amplification products.** The aliquoted reagents must be used the same day the aliquoting was performed. DO NOT store reagents in the aliquot conical tubes.
3. A color change will occur in the reaction tube after the addition of each of the following reagents: Amplification, Enzyme, Probe, and Selection.

E. RUN CONFIGURATION

1. Each worklist must have a set of calibrators at the beginning.
2. For the PROCLEIX ULTRIO Assay and Discriminatory Assays, a set of calibrators consists of one vial each of Negative Calibrator,

HIV-1 Positive Calibrator, HCV Positive Calibrator, and HBV Positive Calibrator.

F. WORK FLOW

1. To minimize the possibility of laboratory areas from becoming contaminated with amplicon, the laboratory area should be arranged with a uni-directional workflow. Proceed from reagent preparation to Sample Preparation to Amplification and then to Detection areas. Samples, equipment, and reagents should not be returned to the area where a previous step was performed. Also, personnel may not move from the dedicated Hybridization Protection Assay (HPA) area back into previous work areas without proper anti-contamination safeguards.
2. Perform reagent preparation in a clean (amplicon- and template-free) area.
3. Perform Sample Preparation, Target Capture, and Pre-Amplification steps in an amplicon-free area.
4. Perform Hybridization Protection Assay in an area separate from the reagent preparation and amplification areas.
5. After pipetting specimens (individual or pooled) into TTUs, remove the TTUs from the deck and load them into a TTU rack. If the same specimens will be tested with a different PROCLEIX® Assay, the specimens may be left on the deck, but the empty calibrator tubes and TCR trough must be discarded. Change gloves after discarding the used calibrator tubes and TCR trough, then load new TTUs into the TTU carriers. See PRECAUTIONS, step B for additional information.

G. ENVIRONMENTAL CONDITIONS

1. The Target Capture, Amplification, Hybridization, and Selection steps are temperature dependent. Therefore, it is imperative that the water baths are maintained within the specified temperature range. Use a calibrated thermometer.
2. Room temperature is defined as 15° to 30°C.
3. Detection is sensitive to temperature. The laboratory temperature in the detection area must be 21° to 27°C.
4. Refer to instrument and software operator's manuals for additional environmental conditions requirements.

H. TIME

The Target Capture, Amplification, and Hybridization Protection Assay steps are all time dependent. Adhere to specific times outlined in ASSAY PROCEDURE.

I. VORTEXING

Proper vortexing is important to the successful performance of the PROCLEIX® ULTRIO® Assay. Vortex equipment speed settings may vary. The vortexer speed should start at a low level and increase until the speed is adequate to achieve the desired results without allowing the reaction mixture to touch the sealing cards. For each step that requires vortexing, it is critical that the contents of the tubes be well-mixed.

J. PIPETTING

1. Operator pipetting precision is critical to assay performance.
2. All pipettors used in the Target Capture, Amplification, and HPA steps must be dedicated to avoid cross-contamination.
3. Take care to deliver reagents, excluding wTCR, to each tube without inserting the pipette tip into the tube or touching the rim of the tube to minimize the chance of carryover from one tube to another.
4. When adding Oil, Probe Reagent, and Selection Reagent, angle the pipette tip toward the sides of the tube, not straight to the bottom, to avoid splashback.

K. MANUAL SPECIMEN PIPETTING

1. When using the manual sample/wTCR pipetting method, improper pipetting technique will affect the results of the assay.
2. In order to avoid the loss of Positive ID Tracking, verification of correct sample ID by a second individual is recommended.
3. Ensure that the TTU is oriented in the rack with the pointed end on the left side and the rounded end on the right side of the rack. Pipette the first calibrator into the first tube next to the pointed end of the TTU. Samples are pipetted from left to right.
4. Use a new pipette tip for each sample and dispose of the tip in a biological waste container after use. Take care to avoid cross-contamination by pipetting the specimens and discarding the used pipette tips without passing over open tubes or touching laboratory surfaces or other pieces of equipment.
5. To avoid the risk of contamination, clean and decontaminate manual sample pipettors between assay runs.
6. Ensure proper sample placement into the correct TTU position as indicated on the manual worklist record.

L. DECONTAMINATION

1. The extremely sensitive nature of the test makes it imperative to take all possible precautions to avoid contamination. Laboratory bench surfaces and pipettes must be decontaminated daily with 0.5% sodium hypochlorite in water (diluted bleach). Allow bleach to contact surfaces for at least 15 minutes and then follow with a water rinse. Chlorine solutions may pit equipment and metal. Thoroughly rinse bleached equipment to avoid pitting.
2. A bleach alternative may be used in Pre-Amplification areas only. Do not use bleach alternatives in Amplification areas or in areas suspected to be contaminated with Amplification products.
3. Reaction tubes must be decontaminated with Deactivation Fluid as described in the PROCLEIX System QRG.
4. Follow instructions provided in the PROCLEIX System QRG for instrument decontamination and maintenance procedures.

M. SEALING CARDS

1. When applying sealing cards, cover the TTUs with the sealing card and press gently to ensure complete contact with all of the tubes. Always use a new sealing card. DO NOT re-use sealing cards.
2. When removing sealing cards, carefully lift and peel in one continuous motion to avoid aerosols and cross-contamination. Immediately dispose of card in appropriate waste container.

ASSAY PROCEDURE

PROCLEIX® ULTRIO® Assay Calibrators and Discriminatory Probe Reagents are to be used with the corresponding master lot of PROCLEIX® ULTRIO® and Discriminatory Assays. The operator must check to ensure that the PROCLEIX ULTRIO Assay Calibrators and Discriminatory Probe Reagents are used with the corresponding master lot of kit reagents as indicated on the PROCLEIX ULTRIO Assay master lot sheet in use.

Specimens from other living donors (except whole blood, blood components, or source plasma) and from cadaveric donors must be tested neat using the individual donor testing method only. If the initial test result from a cadaveric blood specimen is invalid, the specimen may be diluted to overcome potential inhibitory substances as described in SPECIMEN COLLECTION, STORAGE, AND HANDLING, Cadaveric Blood Specimens, J, and retested in singlet.

To run the PROCLEIX ULTRIO Assay for the detection of HIV-1 RNA, HCV RNA, and HBV DNA, follow the steps below for Sample Preparation, Target Capture, Amplification, and the Hybridization Protection Assay. To run the PROCLEIX® Discriminatory Assays for discrimination between HIV-1 RNA, HCV RNA, and HBV DNA, see the

PROCLEIX® HIV-1, HCV, AND HBV DISCRIMINATORY ASSAYS section D, prior to proceeding.

Note: For instrument and software steps, refer to the PROCLEIX® System QRG.

Note: All process steps described below are intended to be completed in a continuous flow with a minimal, if any, delay between steps.

A. SAMPLE PREPARATION/TARGET CAPTURE

Sample Preparation

The PROCLEIX® ULTRIO® Assay has been validated using manual pipetting and a front end pipettor. The use of manual pipetting requires additional operator training and demonstration of proficiency.

IF USING THE MANUAL SAMPLE PIPETTING METHOD:

The repeat pipettors used in these steps must be dedicated for use only in SAMPLE PREPARATION steps.

For sample tracking, an electronic worklist must be created using the PROCLEIX® Worklist Editor software. Refer to the PROCLEIX® System QRG for instructions, or contact Chiron Technical Support. Verification of correct sample ID on the worklist with the specimen tubes and with the detailed assay run report by a second individual is recommended. The assay results within the run report will be marked *M* indicating that the specimens were manually pipetted.

1. Load sufficient Ten Tube Units (TTUs) for the run into a TTU rack.
2. Thoroughly mix the wTCR immediately before use to resuspend microparticles.
3. Refer to the worklist and carefully pipette 400 µL of wTCR to each tube that will contain a sample. To dispense, insert the tip approximately one quarter of the way into the tube at an angle and pipette wTCR down the side of the tube. Take care to avoid touching the rim or the side of the tube with the pipette tip. Always pipette the wTCR first, followed by the sample.
4. Pipette samples.
   a. Refer to the worklist to identify the TTU number with the corresponding calibrator and test specimen identification numbers.
   b. Aspirate 500 µL of each calibrator, external quality control or test specimen from its collection tube using a single channel pipettor with corresponding filtered disposable tip. Insert only the end of the pipette tip into the sample. Do not disturb the sediment, if any.
   c. To dispense, insert the pipette tip halfway into the tube taking care not to touch the sides of the upper half of the tube with the pipette tip. At an angle, pipette the sample down the side of the bottom half of the tube. Hold down the plunger of the pipettor while removing it from the tube. Take care to avoid touching the rim or the side of the tube with the pipette tip when removing it from the tube.
5. Replace the pipette tip with a new tip and repeat step 4 until all samples have been pipetted.
6. Visually inspect tubes to ensure proper sample volume and wTCR volume have been dispensed.
7. Cover the TTUs with sealing cards. See PROCLEIX® SYSTEM USERS, PROCEDURAL NOTES.
8. Proceed to the Target Capture section.

IF USING A FRONT-END PIPETTOR:

1. Prepare front end pipettor for automatic pipetting of calibrators, specimens, and wTCR; refer to the PROCLEIX® System QRG.
2. Instrument will add 400 µL of wTCR to reaction tubes.
3. Instrument will add 500 µL each of calibrators and test specimens into assigned reaction tubes.
4. When all samples have been pipetted, transfer the TTUs to a TTU rack. Cover the TTUs with sealing cards. See PROCLEIX® SYSTEM USERS, PROCEDURAL NOTES on sealing cards.
5. Proceed to the Target Capture section.

Target Capture

1. Vortex the rack of TTUs a minimum of 20 seconds and until magnetic microparticles are resuspended. See PROCLEIX® SYSTEM USERS, PROCEDURAL NOTES on vortexing.
2. The rack may remain at room temperature up to 75 minutes prior to proceeding to the 60° ± 1°C incubation.
3. Incubate the tubes in a water bath at 60° ± 1°C for 20 minutes ± 1 minute.
4. Remove the rack of TTUs and transfer to the Target Capture area.
5. Incubate the rack of TTUs on the lab bench at room temperature for 14 minutes to 20 minutes.
6. Transfer the rack of TTUs to the target capture system (TCS) for 9 to 20 minutes.
7. Carefully remove and dispose of the sealing cards.
8. Perform one Aspiration and Wash step using 1 mL of Wash Solution—refer to the Target Capture section of the PROCLEIX® System QRG for instructions.
9. Cover the TTUs with sealing cards.
10. Vortex to resuspend the microparticle pellets, then inspect the reaction tubes to make sure that all of the magnetic particles have been uniformly suspended.
11. Place the rack of TTUs on the TCS for 4 to 10 minutes.
12. Carefully remove and dispose of the sealing cards.
13. Repeat steps 8 through 12.
14. Completely aspirate the solution from each tube. Refer to the Target Capture section of the PROCLEIX System QRG.
15. Cover the TTUs with sealing cards.
16. Proceed directly to Amplification.

B. AMPLIFICATION

Do not use bleach alternatives in this area.

The repeat pipettors used in these steps must be dedicated for use only in AMPLIFICATION steps.

1. Carefully remove and dispose of the sealing cards.
2. Add 75 µL of Amplification Reagent to each tube (a color change can be observed in the reaction tube). See PROCLEIX® SYSTEM USERS, PROCEDURAL NOTES on pipetting.
3. Add 200 µL of Oil to each tube.
4. Cover the TTUs with sealing cards.
5. Vortex the rack of TTUs a minimum of 20 seconds and until all microparticles are resuspended. Ensure that magnetic particles are no longer adhering to the walls of the tube, and are uniformly resuspended.
6. Incubate the TTUs in a water bath at 60° ± 1°C for 10 minutes ± 1 minute.
7. Incubate the TTUs in a water bath at 41.5° ± 1°C for 9 to 20 minutes.
8. Leaving the rack of TTUs at 41.5° ± 1°C, carefully remove and dispose of the sealing cards. Immediately add 25 µL of the Enzyme Reagent into each tube (a color change can be observed in the reaction tube). Place new sealing cards over the TTUs.
9. Remove the rack of TTUs from the water bath and shake to mix. DO NOT VORTEX. Minimize the time the tubes are out of the water bath.
10. Incubate the rack of TTUs in the water bath at 41.5° ± 1°C for 60 minutes ± 5 minutes.

11. Remove the rack of TTUs from the water bath and transfer it to the Hybridization Protection Assay area. Rack may remain at room temperature for up to 30 minutes prior to the addition of Probe Reagent.

C. HYBRIDIZATION PROTECTION ASSAY (HPA)

A separate, dedicated location for the Hybridization Protection Assay (HPA) step is recommended to minimize amplicon contamination in the assay. This dedicated area should be on a separate bench in a separate area from the reagent and sample preparation and amplification areas. **Do not use bleach alternatives in this area.**

The repeat pipettor used in these steps must be dedicated for use only in HYBRIDIZATION PROTECTION ASSAY steps.

1. Hybridization
   a. Carefully remove and dispose of the sealing cards. See PROCLEIX® SYSTEM USERS, PROCEDURAL NOTES.
   b. Add 100 µL of Probe Reagent into each tube (a color change can be observed in the reaction tube). See PROCLEIX® SYSTEM USERS, PROCEDURAL NOTES on pipetting.
   c. Cover the TTUs with sealing cards. Vortex the rack of TTUs a minimum of 20 seconds and until contents are well-mixed. To avoid possible contamination, do not allow reaction mixture to come in contact with the sealing card. See PROCLEIX® SYSTEM USERS, PROCEDURAL NOTES on vortexing.
   d. Incubate the rack of TTUs in a dedicated water bath at 62° ± 1°C for 15 minutes ± 1 minute.
2. Selection
   a. Remove the rack of TTUs from the 62° ± 1°C water bath. Carefully remove and dispose of the sealing cards.
   b. Add 250 µL of Selection Reagent to each tube (a color change can be observed in the reaction tube).
   c. Cover the TTUs with sealing cards. Vortex the rack of TTUs a minimum of 20 seconds and until contents are well-mixed. To avoid possible contamination, do not allow reaction mixture to come in contact with the sealing card. Return the rack of TTUs to the 62° ± 1°C water bath for 10 minutes ± 1 minute.
   d. Cool the rack of TTUs in a 23° ± 4°C container of water for a minimum of 10 minutes while preparing for Detection.
   e. Remove the rack of TTUs from the 23° ± 4°C container of water onto absorbent material.
3. Detection

   Note: Tube readings should be completed within 75 minutes after completing the selection reaction.

For Detection and decontamination, refer to the PROCLEIX System QRG.

D. PROCLEIX® HIV-1, HCV, AND HBV DISCRIMINATORY ASSAYS

1. To perform the Discriminatory Assays, make the following modifications to the procedure above:
   a. Perform all SAMPLE PREPARATION/TARGET CAPTURE and AMPLIFICATION steps exactly as they are outlined above. Set up separate runs for HIV-1, HCV, and HBV Discriminatory Assays. All three Discriminatory Assays use the same calibrators that are used in the PROCLEIX® ULTRIO® Assay.
   b. Substitute HIV-1, HCV, or HBV Discriminatory Probe Reagent for Probe Reagent when performing HYBRIDIZATION PROTECTION ASSAY.
   c. Choose the appropriate protocol in the luminometer software (refer to the PROCLEIX® System QRG).

QUALITY CONTROL PROCEDURES

I. ACCEPTANCE CRITERIA FOR THE PROCLEIX® ULTRIO® ASSAY AND PROCLEIX® HIV-1, HCV, AND HBV DISCRIMINATORY ASSAYS

A. A run is valid if the minimum number of calibrator replicates is valid and calibrators meet acceptance criteria.
   1. In a PROCLEIX® ULTRIO® Assay run, at least seven of the nine calibrator replicates must be valid. At least two of the three Negative Calibrator replicates and five of the six Positive Calibrator replicates must be valid.
   2. In a PROCLEIX® HIV-1, HCV, or HBV Discriminatory Assay run, at least seven of the 10 calibrator replicates must be valid. In addition, at least two of the three Negative Calibrator replicates must be valid, and the Positive Calibrator criteria below must be met:
      a. For the HIV-1 Discriminatory Assay, two of the three HIV-1 Positive Calibrator replicates must be valid.
      b. For the HCV Discriminatory Assay, two of the three HCV Positive Calibrator replicates must be valid.
      c. For the HBV Discriminatory Assay, two of the three HBV Positive Calibrator replicates must be valid.
   3. The luminometer software will automatically invalidate the run if less than the minimum number of calibrator replicates is valid. All specimens in an invalid run due to calibrators must be retested.
   4. In a valid run, Cutoff values will be automatically calculated for Internal Control (flasher) and Analyte (glower).
   5. In a valid run, specimens with an Analyte Signal (glower signal) greater than the Analyte Cutoff are not invalidated even if the Internal Control signal is below the cutoff. Specimens with an IC signal above 550,000 RLU are invalidated by the software and the reactive status cannot be assessed. Positive Calibrators with an IC signal above 475,000 RLU are invalidated by the software.

B. An assay run or an individual sample may also be invalidated by an operator if specific technical/operator/instrument difficulties were observed and documented. If individual samples in a run are invalidated by an operator, then the percent invalid rate must be manually recalculated.

C. For each run, an alert prints on the run report when more than 10% of the calibrators and specimens in a run are invalid (see the PROCLEIX® System QRG for details). Specimens that are invalid solely due to insufficient sample or wTCR are not included in the calculation of the 10% invalid rate.

D. For runs that exceed the 10% invalid rate, further evaluation of the run is recommended. Review package insert procedures to identify operator errors. In addition, the run report should be reviewed using the criteria described below:
   1. If the invalid specimens are all from the same TTU, those specimens contributing to the 10% invalid rate may have been inadequately washed, or erroneous reagent addition may have occurred. All nonreactive and invalid specimens in the affected TTU must be retested.
   2. If the invalid specimens are randomly located throughout the run, a specific cause that explains the invalid results can be identified, and the remaining valid results have consistent Internal Control RLU values, only the invalid specimens must be retested.
   3. If the invalid specimens are randomly located throughout the run and no specific cause can be identified, all of the nonreactive and invalid specimens in the run must be retested.

Note: Specimens with an overall interpretation of Reactive, as determined by the software, must become the test of record. The

specimens should be resolved according to the resolution algorithm for the reactive specimens, as explained in the INTERPRETATION OF RESULTS section.

II. ACCEPTANCE CRITERIA FOR CALIBRATION AND CALCULATION OF CUTOFF

A. PROCLEIX® ULTRIO® Assay

Negative Calibrator Acceptance Criteria

The Negative Calibrator (NC) is run in triplicate. Each individual Negative Calibrator replicate must have an Internal Control (IC) value greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. Each individual Negative Calibrator replicate must also have an analyte value less than or equal to 45,000 RLU and greater than or equal to 0 RLU. If one of the Negative Calibrator replicate values is invalid due to an IC value or an analyte value outside of these limits, the Negative Calibrator mean (NC_x) will be recalculated based upon the two acceptable values. The run is invalid and must be repeated if two or more of the three Negative Calibrator replicate values have IC values or analyte values that are outside of these limits.

Determination of the mean of the Negative Calibrator values (NC_x) for Internal Control [NC_x (Internal Control)]

Example:

Negative Calibrator		Internal Control RLU
1		124,000
2		126,000
3		125,000
Total Internal Control RLU	=	375,000

$$NC_x\,(\text{Internal Control}) = \frac{\text{Total Internal Control RLU}}{3} = 125{,}000$$

Determination of the mean of the Negative Calibrator values (NC_x) for Analyte [NC_x (Analyte)]

Example:

Negative Calibrator		Analyte RLU
1		14,000
2		16,000
3		15,000
Total Analyte RLU	=	45,000

$$NC_x\,(\text{Analyte}) = \frac{\text{Total Analyte RLU}}{3} = 15{,}000$$

HIV-1 Positive Calibrator Acceptance Criteria

The HIV-1 Positive Calibrator is run in duplicate in the PROCLEIX® ULTRIO® Assay. Individual HIV-1 Positive Calibrator (PC) Analyte values must be less than or equal to 1,800,000 RLU and greater than or equal to 300,000 RLU. IC values may not exceed 475,000 RLU. If one of the HIV-1 Positive Calibrator values is outside these limits, the HIV-1 Positive Calibrator mean (HIV-1 PC_x) will be the remaining acceptable HIV-1 Positive Calibrator value. The run is invalid and must be repeated if both of the HIV-1 Positive Calibrator Analyte values are outside of these limits.

Determination of the mean of the HIV-1 Positive Calibrator (HIV-1 PC_x) values for Analyte [HIV-1 PC_x (Analyte)]

Example:

HIV-1 Positive Calibrator		Analyte RLU
1		690,000
2		700,000
Total Analyte RLU	=	1,390,000

$$\text{HIV-1 } PC_x\,(\text{Analyte}) = \frac{\text{Total Analyte RLU}}{2} = 695{,}000$$

HCV Positive Calibrator Acceptance Criteria

The HCV Positive Calibrator is run in duplicate in the PROCLEIX® ULTRIO® Assay. Individual HCV Positive Calibrator (PC) Analyte values must be less than or equal to 1,000,000 RLU and greater than or equal to 200,000 RLU. IC values may not exceed 475,000 RLU. If one of the HCV Positive Calibrator values is outside these limits, the HCV Positive Calibrator mean (HCV PC_x) will be the remaining acceptable HCV Positive Calibrator value. The run is invalid and must be repeated if both of the HCV Positive Calibrator Analyte values are outside these limits.

Determination of the mean of the HCV Positive Calibrator values (HCV PC_x) for Analyte [HCV PC_x (Analyte)]

Example:

HCV Positive Calibrator		Analyte RLU
1		350,000
2		360,000
Total Analyte RLU	=	710,000

$$\text{HCV } PC_x\,(\text{Analyte}) = \frac{\text{Total Analyte RLU}}{2} = 355{,}000$$

HBV Positive Calibrator Acceptance Criteria

The HBV Positive Calibrator is run in duplicate in the PROCLEIX® ULTRIO® Assay. Individual HBV Positive Calibrator (PC) Analyte values must be less than or equal to 1,800,000 RLU and greater than or equal to 300,000 RLU. IC values may not exceed 475,000 RLU. If one of the HBV Positive Calibrator values is outside these limits, the HBV Positive Calibrator mean (HBV PC_x) will be the remaining acceptable HBV Positive Calibrator value. The run is invalid and must be repeated if both of the HBV Positive Calibrator Analyte values are outside these limits.

Determination of the mean of the HBV Positive Calibrator values (HBV PC_x) for Analyte [HBV PC_x (Analyte)]

Example:

HBV Positive Calibrator		Analyte RLU
1		690,000
2		700,000
Total Analyte RLU	=	1,390,000

$$\text{HBV } PC_x\,(\text{Analyte}) = \frac{\text{Total Analyte RLU}}{2} = 695{,}000$$

Calculation of the Internal Control Cutoff Value

Internal Control Cutoff Value = 0.5 x [NC_x (Internal Control)]

Using values given in the Negative Calibrator example above:

Internal Control Cutoff Value = 0.5 x (125,000)

Internal Control Cutoff Value = 62,500 RLU

Calculation of the HIV-1/HCV/HBV Analyte Cutoff Value

Analyte Cutoff Value = NC_x (Analyte) + [0.02 x HIV-1 PC_x (Analyte)] + [0.04 x HCV PC_x (Analyte)] + [0.02 x HBV PC_x (Analyte)]

Using values given in the Negative Calibrator and Positive Calibrator examples above:

Analyte Cutoff Value = 15,000 + (0.02 x 695,000) + (0.04 x 355,000) + (0.02 x 695,000)

Analyte Cutoff Value = 57,000 RLU

Summary of Acceptance Criteria for PROCLEIX® ULTRIO® Assay

Acceptance Criteria:	
Negative Calibrator	
Analyte	≥ 0 and ≤ 45,000 RLU
Internal Control	≥ 75,000 and ≤ 375,000 RLU
HIV-1 Positive Calibrator	
Analyte	≥ 300,000 and ≤ 1,800,000 RLU
Internal Control	≤ 475,000 RLU
HCV Positive Calibrator	
Analyte	≥ 200,000 and ≤ 1,000,000 RLU
Internal Control	≤ 475,000 RLU
HBV Positive Calibrator	
Analyte	≥ 300,000 and ≤ 1,800,000 RLU
Internal Control	≤ 475,000 RLU

Summary of Cutoff Calculations for PROCLEIX® ULTRIO® Assay

Analyte Cutoff =	NC Analyte Mean RLU + 0.02 x (HIV-1 PC Analyte Mean RLU) + 0.04 x (HCV PC Analyte Mean RLU) + 0.02 x (HBV PC Analyte Mean RLU)
Internal Control Cutoff =	0.5 x (Negative Calibrator IC Mean RLU)

B. PROCLEIX® HIV-1 Discriminatory Assay

Negative Calibrator Acceptance Criteria

The Negative Calibrator must be run in triplicate. Each individual Negative Calibrator (NC) replicate must have an Internal Control (IC) value greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. Each individual Negative Calibrator replicate must also have an analyte value less than or equal to 45,000 RLU and greater than or equal to 0 RLU. If one of the Negative Calibrator replicate values is invalid due to an IC value or analyte value that is outside of these limits, the Negative Calibrator mean (NC_x) will be recalculated based upon the two acceptable values. The run is invalid and must be repeated if two or more of the three Negative Calibrator replicate values have IC values or analyte values that are outside of these limits.

Determination of the mean of the Negative Calibrator (NC_x) values for Internal Control [NC_x (Internal Control)]

Example:

Negative Calibrator		Internal Control RLU
1		124,000
2		126,000
3		125,000
Total Internal Control RLU	=	375,000

$$NC_x\,(\text{Internal Control}) = \frac{\text{Total Internal Control RLU}}{3} = 125{,}000$$

Determination of the mean of the Negative Calibrator values (NC_x) for Analyte [NC_x (Analyte)]

Example:

Negative Calibrator		Analyte RLU
1		12,000
2		11,000
3		13,000
Total Analyte Control RLU	=	36,000

$$NC_x\,(\text{Analyte}) = \frac{\text{Total Analyte RLU}}{3} = 12{,}000$$

HIV-1 Positive Calibrator Acceptance Criteria

The HIV-1 Positive Calibrator is run in triplicate in the PROCLEIX® HIV-1 Discriminatory Assay. Individual HIV-1 Positive Calibrator (PC) Analyte values must be less than or equal to 1,800,000 RLU and greater than or equal to 300,000 RLU. IC values may not exceed 475,000 RLU. If one of the HIV-1 Positive Calibrator values is outside these limits, the HIV-1 Positive Calibrator mean (HIV-1 PC_x) will be recalculated based upon the two acceptable HIV-1 Positive Calibrator values. The run is invalid and must be repeated if more than one of the three HIV-1 Positive Calibrator Analyte values is outside of these limits.

Determination of the mean of the HIV-1 Positive Calibrator (HIV-1 PC_x) values for Analyte [HIV-1 PC_x (Analyte)]

Example:

HIV-1 Positive Calibrator	Analyte RLU
1	1,000,000
2	1,100,000
3	1,050,000
Total Analyte RLU =	3,150,000

$$\text{HIV-1 } PC_x\,(\text{Analyte}) = \frac{\text{Total Analyte RLU}}{3} = 1{,}050{,}000$$

HCV Positive Calibrator and HBV Positive Calibrator Acceptance Criteria

The HCV Positive Calibrator and HBV Positive Calibrator are run in duplicate in the PROCLEIX® HIV-1 Discriminatory Assay on the PROCLEIX® System only. Each individual HCV Positive Calibrator and HBV Positive Calibrator replicate must have an analyte value less than or equal to 45,000 RLU and greater than or equal to 0 RLU. Each HCV Positive Calibrator and HBV Positive Calibrator must also have IC values greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. The run is invalid and must be repeated if more than one of the four calibrator values have IC values or analyte values that are outside these limits.

Calculation of the Internal Control Cutoff Value

Internal Control Cutoff Value = 0.5 x [NC_x (Internal Control)]

Using values given in the Negative Calibrator example above:

Internal Control Cutoff Value = 0.5 x (125,000)

Internal Control Cutoff Value = 62,500 RLU

Calculation of the Analyte Cutoff Value

Analyte Cutoff Value = NC_x (Analyte) + [0.04 x HIV-1 PC_x (Analyte)]

Using values given in the Negative Calibrator and HIV-1 Positive Calibrator examples above:

Analyte Cutoff Value = 12,000 + (0.04 x 1,050,000)

Analyte Cutoff Value = 54,000 RLU

Summary of Acceptance Criteria for the PROCLEIX® HIV-1 Discriminatory Assay

Acceptance Criteria:			
Negative Calibrator			
Analyte	≥ 0	and	≤ 45,000 RLU
Internal Control	≥ 75,000	and	≤ 375,000 RLU
HIV-1 Positive Calibrator			
Analyte	≥ 300,000	and	≤ 1,800,000 RLU
Internal Control	≤ 475,000 RLU		
HCV Positive Calibrator and HBV Positive Calibrator*			
Analyte	≥ 0	and	≤ 45,000 RLU
Internal Control	≥ 75,000	and	≤ 375,000 RLU

***Note that the HCV Positive Calibrator and HBV Positive Calibrator perform similarly to the Negative Calibrator in the PROCLEIX HIV-1 Discriminatory Assay.**

Summary of Cutoff Calculations for the PROCLEIX® HIV-1 Discriminatory Assay

Analyte Cutoff =	NC Analyte Mean RLU + 0.04 x (HIV-1 PC Analyte Mean RLU)
Internal Control Cutoff =	0.5 x (Negative Calibrator IC Mean RLU)

C. PROCLEIX® HCV Discriminatory Assay

Negative Calibrator Acceptance Criteria

The Negative Calibrator must be run in triplicate. Each individual Negative Calibrator replicate must have an Internal Control (IC) value greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. Each individual Negative Calibrator replicate must also have an analyte value less than or equal to 45,000 RLU and greater than or equal to 0 RLU. If one of the Negative Calibrator replicate values is invalid or an IC or analyte value is outside of these limits, the Negative Calibrator mean (NC_x) will be recalculated based upon the two acceptable values. The run is invalid and must be repeated if two or more of the three Negative Calibrator replicate values have IC values or analyte values that are outside of these limits.

Determination of the mean of the Negative Calibrator (NC_x) values for Internal Control [NC_x (Internal Control)]

Example:

Negative Calibrator	Internal Control RLU
1	124,000
2	126,000
3	125,000
Total Internal Control RLU =	375,000

$$NC_x\,(\text{Internal Control}) = \frac{\text{Total Internal Control RLU}}{3} = 125{,}000$$

Determination of the Analyte mean of the Negative Calibrator values (NC_x) for Analyte [NC_x (Analyte)]

Example:

Negative Calibrator	Analyte RLU
1	20,000
2	22,000
3	18,000
Total Analyte RLU =	60,000

$$NC_x\,(\text{Analyte}) = \frac{\text{Total Analyte RLU}}{3} = 20{,}000$$

HCV Positive Calibrator Acceptance Criteria

The HCV Positive Calibrator is run in triplicate in the PROCLEIX® HCV Discriminatory Assay. Individual HCV Positive Calibrator values must be less than or equal to 2,700,000 RLU and greater than or equal to 400,000 RLU. IC values may not exceed 475,000 RLU. If one of the HCV Positive Calibrator values is outside these limits, the HCV Positive Calibrator mean (HCV PC_x) will be recalculated based upon the two acceptable HCV Positive Calibrator values. The run is invalid and must be repeated if more than one of the three HCV Positive Calibrator Analyte values is outside of these limits.

Determination of the Analyte mean of the HCV Positive Calibrator values (HCV PC_x) values for Analyte [HCV PC_x (Analyte)]

Example:

HCV Positive Calibrator	Analyte RLU
1	1,300,000
2	1,200,000
3	1,250,000
Total Analyte RLU =	3,750,000

$$\text{HCV } PC_x\,(\text{Analyte}) = \frac{\text{Total Analyte RLU}}{3} = 1{,}250{,}000$$

HIV-1 Positive Calibrator and HBV Positive Calibrator Acceptance Criteria

The HIV-1 Positive Calibrator and the HBV Positive Calibrator are run in duplicate in the PROCLEIX® HCV Discriminatory Assay only. Each individual HIV-1 Positive Calibrator and HBV Positive Calibrator must have analyte values less than or equal to 45,000 RLU and greater than or equal to 0 RLU. Each HIV-1 Positive Calibrator and HBV Positive Calibrator must also have an IC value greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. The run is invalid and must be repeated if more than one of the four calibrator values have IC values or analyte values that are outside of these limits.

Calculation of the Internal Control Cutoff Value

Internal Control Cutoff Value = 0.5 x [NC_x (Internal Control)]

Using values given in the Negative Calibrator example above:

Internal Control Cutoff Value = 0.5 x (125,000)

Internal Control Cutoff Value = 62,500 RLU

Calculation of the Analyte Cutoff Value

Analyte Cutoff Value = NC_x(Analyte) + [0.04 x HCV PC_x(Analyte)]

Using values given in the Negative Calibrator and HCV Positive Calibrator examples above:

Analyte Cutoff Value = 20,000 + (0.04 x 1,250,000)

Analyte Cutoff Value = 70,000 RLU

Summary of Acceptance Criteria for the PROCLEIX® HCV Discriminatory Assay

Acceptance Criteria:			
Negative Calibrator			
Analyte	≥ 0	and	≤ 45,000 RLU
Internal Control	≥ 75,000	and	≤ 375,000 RLU
HIV-1 Positive Calibrator and HBV Positive Calibrator*			
Analyte	≥ 0	and	≤ 45,000 RLU
Internal Control	≥ 75,000	and	≤ 375,000 RLU
HCV Positive Calibrator			
Analyte	≥ 400,000	and	≤ 2,700,000 RLU
Internal Control	≤ 475,000 RLU		

***Note that the HIV-1 Positive Calibrator and HBV Positive Calibrator perform similarly to the Negative Calibrator in the PROCLEIX HCV Discriminatory Assay**

Summary of Cutoff Calculations for the PROCLEIX® HCV Discriminatory Assay

Analyte Cutoff =	NC Analyte Mean RLU + 0.04 x (HCV PC Analyte Mean RLU)
Internal Control Cutoff =	0.5 x (Negative Calibrator IC Mean RLU)

D. PROCLEIX® HBV Discriminatory Assay

Negative Calibrator Acceptance Criteria

The Negative Calibrator must be run in triplicate. Each individual Negative Calibrator (NC) replicate must have an Internal Control (IC) value greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. Each individual Negative Calibrator replicate must also have an analyte value less than or equal to 45,000 RLU and greater than or equal to 0 RLU. If one of the Negative Calibrator replicate values is invalid due to an IC value or analyte value that is outside of these limits, the Negative Calibrator mean (NCx) will be recalculated based upon the two acceptable values. The run is invalid and must be repeated if two or more of the three Negative Calibrator replicate values have IC values or analyte values that are outside of these limits.

Determination of the mean of the Negative Calibrator (NC_x) values for Internal Control [NC_x (Internal Control)]

Example:

Negative Calibrator	Internal Control RLU
1	124,000
2	126,000
3	125,000
Total Internal Control RLU =	375,000

$$NC_x \text{ (Internal Control)} = \frac{\text{Total Internal Control RLU}}{3} = 125{,}000$$

Determination of the mean of the Negative Calibrator values (NC_x) for Analyte [NC_x (Analyte)]

Example:

Negative Calibrator	Analyte RLU
1	12,000
2	11,000
3	13,000
Total Analyte RLU =	36,000

$$NC_x \text{ (Analyte)} = \frac{\text{Total Analyte RLU}}{3} = 12{,}000$$

HBV Positive Calibrator Acceptance Criteria

The HBV Positive Calibrator is run in triplicate in the PROCLEIX® HBV Discriminatory Assay. Individual HBV Positive Calibrator (PC) Analyte values must be less than or equal to 1,800,000 RLU and greater than or equal to 300,000 RLU. IC values may not exceed 475,000 RLU. If one of the HBV Positive Calibrator values is outside these limits, the HBV Positive Calibrator mean will be recalculated based upon the two acceptable HBV Positive Calibrator values. The run is invalid and must be repeated if more than one of the three HBV Positive Calibrator Analyte values is outside of these limits.

Determination of the mean of the HBV Positive Calibrator (HBV PC_x) values for Analyte [HBV PC_x (Analyte)]

Example:

HBV Positive Calibrator	Analyte RLU
1	1,150,000
2	1,160,000
3	1,170,000
Total Analyte RLU =	3,480,000

$$\text{HBV } PC_x \text{ (Analyte)} = \frac{\text{Total Analyte RLU}}{3} = 1{,}160{,}000$$

HIV-1 Positive Calibrator and HCV Positive Calibrator Acceptance Criteria

The HIV-1 Positive Calibrator and the HCV Positive Calibrator are run in duplicate in the PROCLEIX® HBV Discriminatory Assay only. Each individual HIV-1 Positive Calibrator and HCV Positive Calibrator replicate must have an analyte value less than or equal to 45,000 RLU and greater than or equal to 0 RLU. Each HIV-1 Positive Calibrator and HCV Positive Calibrator must also have IC values greater than or equal to 75,000 RLU and less than or equal to 375,000 RLU. The run is invalid and must be repeated if more than one of the four calibrator values have IC values or analyte values that are outside these limits.

Calculation of the Internal Control Cutoff Value

Internal Control Cutoff Value = 0.5 x [NC_x (Internal Control)]

Using values given in the Negative Calibrator example above:

Internal Control Cutoff Value = 0.5 x (125,000)

Internal Control Cutoff Value = 62,500 RLU

Calculation of the Analyte Cutoff Value

Analyte Cutoff Value = NC_x (Analyte) + [0.04 x HBV PC_x (Analyte)]

Using values given in the Negative Calibrator and HBV Positive Calibrator examples above:

Analyte Cutoff Value = 12,000 + (0.04 x 1,160,000)

Analyte Cutoff Value = 58,400 RLU

Summary of Acceptance Criteria for the PROCLEIX® HBV Discriminatory Assay

Acceptance Criteria:			
Negative Calibrator			
Analyte	≥ 0	and	≤ 45,000 RLU
Internal Control	≥ 75,000	and	≤ 375,000 RLU
HBV Positive Calibrator			
Analyte	≥ 300,000	and	≤ 1,800,000 RLU
Internal Control	≤ 475,000 RLU		
HIV-1 Positive Calibrator and HCV Positive Calibrator*			
Analyte	≥ 0	and	≤ 45,000 RLU
Internal Control	≥ 75,000	and	≤ 375,000 RLU

***Note that the HIV-1 Positive Calibrator and HCV Positive Calibrator perform similarly to the Negative Calibrator in the PROCLEIX HBV Discriminatory Assay.**

Summary of Cutoff Calculations for the PROCLEIX® HBV Discriminatory Assay

Analyte Cutoff =	NC Analyte Mean RLU + 0.04 x (HBV PC Analyte Mean RLU)
Internal Control Cutoff =	0.5 x (Negative Calibrator IC Mean RLU)

INTERPRETATION OF RESULTS

All calculations described above are performed by the luminometer software. Two cutoffs are determined for each assay: one for the Analyte Signal (glower signal), termed the Analyte cutoff, and one for the Internal Control Signal (flasher signal), termed the Internal Control Cutoff. The calculation of these cutoffs is shown above. For each sample, an Analyte Signal RLU value and Internal Control Signal RLU value are determined. Analyte Signal RLU divided by the Analyte Cutoff is abbreviated as the Analyte Signal/cutoff (S/CO) on the report.

A specimen is considered Nonreactive if the Analyte Signal is less than the Analyte Cutoff (i.e., Analyte S/CO <1.00) and the Internal Control (IC) Signal is greater than or equal to the Internal Control Cutoff (IC Cutoff) and less than or equal to 550,000 RLU. A specimen is considered Reactive if the Analyte Signal is greater than or equal to the Analyte Cutoff (i.e., Analyte S/CO ≥1.00) and the Internal Control Signal is less than or equal to 550,000 RLU. Reactive results will be designated by the software. A specimen is considered Invalid if the Analyte Signal is less than the Analyte Cutoff (i.e., Analyte S/CO <1.00) and the Internal Control Signal is less than the Internal Control Cutoff. A specimen is also considered Invalid if the Internal Control Signal is greater than 550,000 RLU.

Cadaveric blood specimens, when tested neat, may be invalid due to inhibitory substances within the specimen. These invalid specimens may be diluted as in SPECIMEN COLLECTION, STORAGE, AND HANDLING, Cadaveric Blood Specimens, step J, and retested in singlet.

Summary of Specimen Interpretation:

Specimen Interpretation	Criteria
Nonreactive	Analyte S/CO <1.00 and IC ≥ IC Cutoff and IC ≤ 550,000 RLU
Reactive	Analyte S/CO ≥ 1.00 and IC ≤ 550,000 RLU*
Invalid	IC > 550,000 RLU or Analyte S/CO <1.00 and IC < IC Cutoff

*For specimens with IC signal greater than 550,000 RLU, the specimen will be invalidated by the software and the reactive status cannot be assessed.

1. Any specimen with an overall interpretation of Invalid in the PROCLEIX® ULTRIO® Assay, PROCLEIX® HIV-1 Discriminatory Assay, PROCLEIX® HCV Discriminatory Assay, or PROCLEIX® HBV Discriminatory Assay must be retested in the same assay in singlet, except as noted in step 8. Cadaveric specimens with an overall interpretation of Invalid in the PROCLEIX® ULTRIO® Assay, PROCLEIX® HIV-1 Discriminatory Assay, PROCLEIX® HCV Discriminatory Assay, or PROCLEIX® HBV Discriminatory Assay previously diluted 1:5 may be retested in singlet, diluted at the 1:5 dilution, except as noted in step 8.
2. If at any point in the testing algorithm there is insufficient volume to complete the testing then an alternate specimen from the index donation (e.g., plasma unit or serology tube) may be used as long as the storage criteria in the package insert are met.
3. Specimens with a valid Internal Control value and with an Analyte S/CO less than 1.00 in the PROCLEIX ULTRIO Assay are considered nonreactive for HIV-1 RNA, HCV RNA, and HBV DNA. If the nonreactive specimen is a pool, each of the individual specimens comprising the pool is considered nonreactive and no further testing is required.
4. Specimens with an Analyte S/CO greater than or equal to 1.00 and an IC Signal less than or equal to 550,000 RLU are considered **Reactive**.
5. IF THE REACTIVE SPECIMEN IS A POOL, then each of the individual specimens comprising the pool must be tested with the PROCLEIX ULTRIO Assay.
    a. If an individual specimen tests nonreactive with the PROCLEIX ULTRIO Assay, then the specimen is considered nonreactive for HIV-1 RNA, HCV RNA, and HBV DNA and no further testing is required.
    b. If an individual specimen tests Reactive with the PROCLEIX ULTRIO Assay, then the specimen must be tested with the HIV-1 Discriminatory, HCV Discriminatory, and HBV Discriminatory Assays.
        (1) If an individual specimen then tests Reactive with one or more Discriminatory tests, then the specimen is considered Reactive-Discriminated.
        (2) If an individual specimen then tests nonreactive with all Discriminatory tests, then the specimen is considered Non-Discriminated.
6. IF THE REACTIVE SPECIMEN IS FROM AN INDIVIDUAL DONATION FROM A DONOR OF WHOLE BLOOD, BLOOD COMPONENTS OR SOURCE PLASMA, then the specimen must be tested with the HIV-1 Discriminatory, HCV Discriminatory, and HBV Discriminatory Assays.

a. If an individual specimen then tests Reactive with one or more Discriminatory tests, then the specimen is considered Reactive-Discriminated.

b. If an individual specimen then tests nonreactive with all Discriminatory tests, then the specimen is considered Non-Discriminated. Caution: Some HBV true positive specimens reactive by PROCLEIX ULTRIO Assay individual donation screening may test nonreactive by the PROCLEIX HBV Discriminatory Assay. (See statement in LIMITATIONS OF THE PROCEDURE.)

7. IF THE REACTIVE SPECIMEN IS FROM AN INDIVIDUAL DONATION FROM ANY OTHER LIVING DONOR (I.E., NOT A BLOOD DONOR) OR FROM A CADAVERIC DONOR, then the specimen must be tested with the HIV-1 Discriminatory, HCV Discriminatory, and HBV Discriminatory Assays.

   a. If an individual specimen then tests Reactive with one or more Discriminatory tests, then the specimen is considered Reactive-Discriminated.

   b. If an individual specimen then tests nonreactive with all Discriminatory tests, then the specimen is considered Non-Discriminated. The Non-Discriminated specimen may be retested in the PROCLEIX ULTRIO Assay if sufficient sample is available.

      (1) If the individual specimen tests nonreactive in the repeated PROCLEIX ULTRIO Assay, then the specimen is considered nonreactive for HIV-1 RNA, HCV RNA, and HBV DNA and no further testing is required.

      (2) If the individual specimen tests Reactive in the repeated PROCLEIX ULTRIO Assay, then the specimen is considered Repeatedly Reactive, Non-Discriminated for HIV-1 RNA, HCV RNA, and HBV DNA. Further clarification of these specimens for informational purposes may be obtained by testing an alternate specimen from the index donation with the PROCLEIX Assays and/or by follow-up testing. Results of testing obtained for clarification do not replace test results for purposes of cell/tissue donor eligibility. Caution: Some HBV true positive specimens reactive by PROCLEIX ULTRIO Assay individual donation screening may test nonreactive by the PROCLEIX HBV Discriminatory Assay. (See statement in LIMITATIONS OF THE PROCEDURE.)

8. Reactive specimens in an operator invalidated run are identified by the luminometer software as reactive, and must become the test of record. Any reactive result serves as the test of record and the sample should be resolved according to the resolution algorithm for reactive specimens, as explained in the INTERPRETATION OF RESULTS section.

9. HIV seroreactive specimens found to be Reactive- HIV-1 Discriminated in the PROCLEIX® Assays may be considered positive for HIV-1 nucleic acid. HCV seroreactive specimens found to be Reactive-HCV Discriminated in the PROCLEIX Assays may be considered positive for HCV nucleic acid. HBV seroreactive specimens found to be Reactive-HBV Discriminated in the PROCLEIX Assays may be considered positive for HBV nucleic acid. The interpretation of Reactive-Discriminated specimen results on specimens that are nonreactive by serology is unclear.

10. Specimens that are Nonreactive in the PROCLEIX ULTRIO Assay or are Reactive in the PROCLEIX ULTRIO Assay but are not HIV-1 Discriminated, and are also repeatedly reactive in a licensed donor screening test for antibodies to HIV-1, should be further tested using an FDA approved HIV-1 supplemental test (such as Western blot or immunofluorescence assay).

    Specimens that are Nonreactive in the PROCLEIX ULTRIO Assay or are Reactive in the PROCLEIX ULTRIO Assay but are not HCV Discriminated, and are also repeatedly reactive in a licensed donor screening test for antibodies to HCV, should be further tested using an FDA approved HCV supplemental test (such as RIBA).

11. Donors with specimens that are reactive in the PROCLEIX HIV-1, HCV, or HBV Discriminatory Assays and/or repeatedly EIA reactive by licensed serological tests for HIV, HCV or HBV (or any combinations of these), should be referred for medical evaluation. A clinical diagnosis can be made only if the person meets the case definition(s) established by the Centers for Disease Control and Prevention.[32, 33]

LIMITATIONS OF THE PROCEDURE

This assay has been approved for use with the PROCLEIX® System only.

The PROCLEIX® ULTRIO® Assay may not be used to replace antibody-detection tests such as an EIA test for HIV-1 or HCV.

The clinical sensitivity for the PROCLEIX ULTRIO Assay has been demonstrated for specimens with HIV-1 or HCV viral RNA concentrations equal to or greater than 100 copies/mL or HBV viral DNA concentrations equal to or greater than 15 IU/mL. Samples with less than these concentrations may not yield reproducible results.

The results of the HBV genotype studies shown in Table 27 indicated equivalent performance of the PROCLEIX HBV Discriminatory Assay to the PROCLEIX ULTRIO Assay at a concentration of 300 copies/mL, but the PROCLEIX HBV Discriminatory Assay was less sensitive than the PROCLEIX ULTRIO Assay at the lower concentrations of 30 and 100 copies/mL. Therefore, some HBV true positive specimens reactive in PROCLEIX ULTRIO Assay individual donation screening may test nonreactive by the PROCLEIX HBV Discriminatory Assay.

Assays must be performed and results interpreted according to procedures provided.

Deviation from these procedures, adverse shipping and/or storage conditions, or use of outdated calibrators and/or reagents may produce unreliable results.

Various donor and donation factors were evaluated for interference and cross-reactivity in the assays. A small portion had unexpected results in greater than 5% of the samples tested (Tables 13 to 16).

GENERAL INFORMATION

PERFORMANCE CHARACTERISTICS

REPRODUCIBILITY

PROCLEIX® SYSTEM

Reproducibility of the PROCLEIX® ULTRIO® Assay, HIV-1 Discriminatory Assay, HCV Discriminatory Assay, and HBV Discriminatory Assay was evaluated at three blood center laboratories. For determination of the reproducibility of each assay, 10 members from a reproducibility panel were tested as individual specimens (Tables 1-4). Eight of the panel members were either positive for HIV-1 RNA (150, 2,500 and 10,000 c/mL), HCV RNA (150 and 2,500 c/mL), and/or HBV DNA (50 and 500 IU/mL) and two panel members were HIV-1, HCV, and HBV negative.

The reproducibility panels were tested by a total of seven operators (two to three from each testing site) with three different Clinical Lots over multiple nonconsecutive days, using an automated front end pipettor (Tecan) or manual pipetting of specimen and working Target Capture Reagent (wTCR). Twenty-four valid runs were generated for each assay across three Clinical Lots, with each panel member tested in triplicate per run and each operator performing testing for at least eight days.

The Reproducibility Study assessed intra- and inter-assay, inter-lot and inter-site variability of the PROCLEIX ULTRIO Assay and each discriminatory assay.

Reproducibility analyses included evaluation of percent agreement and mean Signal/Cutoff (S/CO) ratios for panel members and mean Relative Light Unit (RLU) values for the Negative, HIV-1 Positive, HCV Positive, and HBV Positive Calibrators and evaluation of standard deviation (SD) and percent coefficient of variation (%CV) of those S/CO ratios and RLU values for each of the four variance factors (Tables 1-4). The mean analyte S/CO ratios were analyzed for the positive panel members and the IC S/CO ratios were analyzed for the negative panel members. The mean analyte RLU values were analyzed for the Positive Calibrators and the IC RLU values were analyzed for the Negative Calibrators. The percent agreement between the assay results and the true status of each panel member was calculated using analyte S/CO for all panel members. Since no significant difference in assay reproducibility was observed between automated Tecan pipettor and manual pipetting, results from testing of individual specimens for the two pipetting methods were combined and are shown in the tables below (Tables 1-4).

For the PROCLEIX ULTRIO Assay, results for all individual panel members are shown. For the discriminatory assays, results for negative panel members and panel members containing target(s) that should be nonreactive were combined. Results for panel members containing target that should be reactive are shown individually.

For the PROCLEIX ULTRIO Assay and three discriminatory assays, the overall percent agreement of test results was 100% for positive samples and 98.6 - 100% for negative samples. With regard to signal variability, intra-assay (or random error) and inter-assay factors, in most cases, were the largest and second largest contributors to total variance (according to SD values) in the PROCLEIX ULTRIO Assay, and the PROCLEIX® HIV-1 and HCV Discriminatory Assays. For the PROCLEIX® HBV Discriminatory Assay, the inter-assay factor was the largest contributor to total variance (according to SD values), followed by the intra-assay and inter-site factors, which similarly contributed to total variance. It should be noted that while these factors were responsible for the majority of the variance in the assays, the %CV of each of these components by itself did not exceed 11.2% for any positive or negative samples, in any assay. Therefore, the reproducibility of the assays is robust and the variation that is observed can be attributed primarily to random error. Other variance factors, including testing site and Clinical Lot, have zero or very little impact on assay performance (Tables 1-4).

Table 1. PROCLEIX® System - Reproducibility of the PROCLEIX® ULTRIO® Assay (analysis of analyte signals, unless noted)

Specimen	N	Concentration*	Number of replicates	% Agreement	Mean S/CO	Intra-Assay		Inter-Assay		Inter-Lot		Inter-Site	
						SD	%CV	SD	%CV	SD***	%CV	SD***	%CV
Nonreactive**	1	0	72	98.6	2.00	0.10	4.9	0.08	3.9	0.00	0.0	0.01	0.6
Nonreactive**	1	0	72	100	2.02	0.07	3.4	0.08	4.1	0.00	0.0	0.00	0.0
HIV-1	1	10,000	72	100	17.06	0.48	2.8	0.56	3.3	0.37	2.2	0.00	0.0
HIV-1/ HCV/HBV	1	2,500/2,500/ 500	72	100	37.6	0.84	2.2	1.55	4.1	0.83	2.2	0.42	1.1
HCV	1	150	72	100	6.06	0.27	4.4	0.27	4.5	0.24	3.9	0.30	4.9
HCV/HBV	1	2,500/500	72	100	21.79	0.50	2.3	0.85	3.9	0.55	2.5	0.43	2.0
HIV-1	1	150	72	100	13.72	1.46	10.6	0.54	4.0	0.68	4.9	0.89	6.5
HBV	1	50	72	100	14.92	0.37	2.5	0.55	3.7	0.55	3.7	0.24	1.6
HIV-1/HBV	1	2,500/500	72	100	31.01	0.76	2.4	1.13	3.6	0.55	1.8	0.21	0.7
HIV-1/HCV	1	2,500/2,500	72	100	23.32	0.49	2.1	0.81	3.5	0.00	0.0	0.00	0.0

Specimen	Number of replicates	% Agreement	Mean RLU	Intra-Assay		Inter-Assay		Inter-Lot		Inter-Site	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Calibrator**	71	N/A	216,522	6,392	3.0	8,055	3.7	9,533	4.4	13,466	6.2
HIV-1 Positive Calibrator	47	N/A	1,242,002	16,313	1.3	25,122	2.0	25,142	2.0	49,452	4.0
HCV Positive Calibrator	48	N/A	611,102	17,099	2.8	23,958	3.9	0.00	0.0	37,381	6.1
HBV Positive Calibrator	48	N/A	1,138,995	26,379	2.3	38,770	3.4	30,233	2.7	72,948	6.4

N = Number of panel members combined for this analysis
* Concentration = copies/mL for HIV-1 and HCV, IU/mL for HBV
** Analysis of internal control signal
*** Per NCCLS guidelines, (EP5-A, page 7), numbers <0 are recorded as 0.

Table 2. PROCLEIX® System - Reproducibility of the PROCLEIX® HIV-1 Discriminatory Assay (analysis of analyte signals, unless noted)

Specimen	N	Concentration*	Number of replicates	% Agreement	Mean S/CO	Intra-Assay		Inter-Assay		Inter-Lot		Inter-Site	
						SD	%CV	SD***	%CV	SD***	%CV	SD***	%CV
Nonreactive**	5	0	359	99.7	1.98	0.09	4.5	0.03	1.5	0.03	1.4	0.00	0.0
HIV-1	1	10,000	72	100	25.80	0.66	2.5	0.80	3.1	0.22	0.8	0.00	0.0
HIV-1/ HCV/HBV	1	2,500/2,500/ 500	72	100	24.52	0.54	2.2	0.61	2.5	0.00	0.0	0.00	0.0
HIV-1	1	150	72	100	20.51	2.19	10.7	0.42	2.1	0.66	3.2	0.61	3.0
HIV-1/HBV	1	2,500/500	72	100	24.57	0.61	2.5	0.85	3.4	0.31	1.3	0.00	0.0
HIV-1/HCV	1	2,500/2,500	72	100	24.1	1.86	7.7	0.00	0.0	0.00	0.0	0.51	2.1

Specimen	Number of replicates	% Agreement	Mean RLU	Intra-Assay		Inter-Assay		Inter-Lot		Inter-Site	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Calibrator**, ****	166	N/A	220,588	6,334	2.9	9,884	4.5	14,777	6.7	8,168	3.7
HIV-1 Positive Calibrator	71	N/A	1,252,970	31,621	2.5	34,260	2.7	18,887	1.5	86,575	6.9

N = Number of panel members combined for this analysis
* Concentration = copies/mL for HIV-1 and HCV, IU/mL for HBV. For nonreactive specimens, only the HIV-1 concentration is listed.
** Analysis of internal control signal
*** Per NCCLS guidelines, (EP5-A, page 7), numbers <0 are recorded as 0.
**** Analysis of Negative Calibrator and HBV and HCV Positive Calibrators

Table 3. PROCLEIX® System - Reproducibility of the PROCLEIX® HCV Discriminatory Assay (analysis of analyte signals, unless noted)

Specimen	N	Concentration*	Number of replicates	% Agreement	Mean S/CO	Intra-Assay		Inter-Assay		Inter-Lot		Inter-Site	
						SD	%CV	SD	%CV	SD***	%CV	SD***	%CV
Nonreactive**	6	0	413	99.0	2.07	0.08	4.0	0.09	4.2	0.01	0.7	0.00	0.0
HIV-1/ HCV/HBV	1	2,500/2,500/ 500	69	100	22.18	0.50	2.3	1.27	5.7	0.21	0.9	0.42	1.9
HCV	1	150	69	100	19.08	0.98	5.1	1.01	5.3	0.48	2.5	0.00	0.0
HCV/HBV	1	2,500/500	69	100	22.33	0.58	2.6	1.17	5.3	0.28	1.2	0.59	2.7
HIV-1/HCV	1	2,500/2,500	68	100	21.88	2.45	11.2	1.04	4.8	0.00	0.0	0.57	2.6
Specimen			Number of replicates	% Agreement	Mean RLU	Intra-Assay		Inter-Assay		Inter-Lot		Inter-Site	
						SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Calibrator**, ****			113	N/A	220,270	7,527	3.4	9,308	4.2	19,184	8.7	13,180	6.0
HCV Positive Calibrator			69	N/A	1,318,289	26,043	2.0	54,331	4.1	42,644	3.2	79,828	6.1

N = Number of panel members combined for this analysis
* Concentration = copies/mL for HIV-1 and HCV, IU/mL for HBV. For nonreactive specimens, only the HCV concentration is listed.
** Analysis of internal control signal
*** Per NCCLS guidelines, (EP5-A, page 7), numbers <0 are recorded as 0.
**** Analysis of Negative Calibrator and HIV-1 and HBV Positive Calibrators

Table 4. PROCLEIX® System - Reproducibility of the PROCLEIX® HBV Discriminatory Assay (analysis of analyte signals, unless noted)

Specimen	N	Concentration*	Number of replicates	% Agreement	Mean S/CO	Intra-Assay		Inter-Assay		Inter-Lot		Inter-Site	
						SD	%CV	SD	%CV	SD***	%CV	SD***	%CV
Nonreactive**	6	0	430	99.8	2.00	0.12	5.8	0.10	4.9	0.02	0.9	0.00	0.0
HIV-1/ HCV/HBV	1	2,500/2,500/ 500	72	100	25.17	0.57	2.3	1.55	6.2	0.00	0.0	0.66	2.6
HCV/HBV	1	2,500/500	72	100	25.14	0.66	2.6	1.50	6.0	0.03	0.1	0.69	2.7
HBV	1	50	72	100	25.55	0.69	2.7	1.62	6.3	0.00	0.0	0.92	3.6
HIV-1/HBV	1	2,500/500	72	100	26.06	0.69	2.7	1.79	6.9	0.00	0.0	0.78	3.0
Specimen			Number of replicates	% Agreement	Mean RLU	Intra-Assay		Inter-Assay		Inter-Lot		Inter-Site	
						SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Calibrator**, ****			119	N/A	207,762	7,641	3.7	10,093	4.9	18,207	8.8	10,636	5.1
HBV Positive Calibrator			72	N/A	1,083,154	32,781	3.0	40,616	3.8	39,069	3.6	68,573	6.3

N = Number of panel members combined for this analysis
* Concentration = copies/mL for HIV and HCV, IU/mL for HBV. For nonreactive specimens, only the HBV concentration is listed.
** Analysis of internal control signal
*** Per NCCLS guidelines, (EP5-A, page 7), numbers <0 are recorded as 0.
**** Analysis of Negative Calibrator and HIV-1 and HCV Positive Calibrators

SPECIFICITY IN NORMAL BLOOD DONORS

PROCLEIX® SYSTEM

The clinical specificity of the PROCLEIX® ULTRIO® Assay was determined on the PROCLEIX® System in 16-sample pools made from plasma from either whole blood donations or paid source plasma (PSP) donors and in individual donor samples (IDS) from whole blood donations. The clinical specificity of the HIV-1, HCV, and HBV Discriminatory Assays was determined on the PROCLEIX System in IDS from whole blood donations.

The study was conducted at three blood center testing laboratories and one source plasma center using plasma samples derived from approximately 32 geographically diverse blood donor sites in the United States. During the study, all testing was performed linked using three Clinical Lots of PROCLEIX ULTRIO Assay reagent kits. All of the samples collected for the study were tested with the PROCLEIX ULTRIO Assay, with the licensed PROCLEIX® HIV-1/HCV Assay and with licensed HBsAg serologic tests and, as appropriate, confirmatory tests.

Specificity of the PROCLEIX ULTRIO Assay was calculated from 12,028 16-sample plasma pools from whole blood donations, 12,780 IDS from whole blood donations, and 1,198 16-sample plasma pools from PSP donors (Table 7). Specificity of the HIV-1 (n=1,797), HCV (n=1,810), and HBV (n=1,795) Discriminatory Assays was calculated using results of IDS from whole blood donations. Voluntary source plasma (VSP) donations were also collected and included in either the 16-sample pools or IDS from whole blood donations (n=303 and 9, respectively) tested in the PROCLEIX ULTRIO Assay. In the specificity evaluation, the results from the PROCLEIX ULTRIO Assay and the associated Discriminatory Assays were compared to results from the licensed PROCLEIX HIV-1/HCV Assay and associated Discriminatory Assays and to results from licensed HBsAg serologic tests. The specificity was also based on the results from alternate licensed or validated nucleic acid tests (Alternate NAT), which were performed on IDS or individual samples from pools with discordant results.

Rates of PROCLEIX ULTRIO Assay reactivity are presented in Tables 5 and 6 for pools and IDS from whole blood donations that were included in the clinical specificity analyses. The overall clinical specificity results are summarized in Table 7. Table 8 shows clinical specificity by site for pools and IDS from whole blood donations. Pools from PSP donations were tested at only one site.

Table 5. Clinical Specificity Study: PROCLEIX® ULTRIO® Assay Reactivity in 16-Sample Pools

Results	n	Percent (95% CI)
Total pools tested	12,028	100.00%
Nonreactive pools	11,796	98.07% (97.81-98.31%)
Initially reactive pools	232	1.93% (1.69-2.19%)
Pool, individual constituent(s), and reference test reactive (true positive)	167	1.39% (1.19-1.61%)
Pool, individual constituent(s), and discriminatory assay reactive, reference test nonreactive, Alternate NAT reactive (true positive)	6	0.05% (0.02-0.11%)
Pool reactive, individual constituents and reference test nonreactive (false positive)	50	0.42% (0.31-0.55%)
Pool, individual constituent(s), and discriminatory assay reactive, reference test nonreactive, Alternate NAT not available (false positive)	2	0.02% (0.00-0.06%)
Pool and individual constituent(s) reactive, discriminatory assay, individual constituent(s) retest, and reference test nonreactive (false positive)	7	0.06% (0.02-0.12%)

Table 6. Clinical Specificity Study: PROCLEIX® ULTRIO® Assay Reactivity in IDS

Results	n	Percent (95% CI)
Total IDS tested	12,780	100.00%
Nonreactive IDS	12,480	97.65% (97.38-97.91%)
Initially reactive IDS	300	2.35% (2.09-2.62%)
IDS and reference test reactive (true positive)	186	1.46% (1.25-1.68%)
IDS and discriminatory assay reactive, reference test nonreactive, and Alternate NAT reactive (true positive)	7	0.05% (0.02-0.11%)
IDS and discriminatory assay reactive, reference test nonreactive, Alternate NAT nonreactive or unavailable (false positive)	10*	0.08% (0.04-0.14%)
IDS reactive, discriminatory assay, retest, and reference test nonreactive (false positive)	96**	0.75% (0.61-0.92%)
IDS reactive, discriminatory assay nonreactive, retest reactive, and reference test nonreactive (false positive)	1	0.01% (0.00-0.04%)

* Eight IDS had unavailable Alternate NAT results because they were invalidated (n=7) or for other reasons. Two IDS were Alternate NAT nonreactive.
** Includes four initially reactive IDS without discriminatory assay results and with nonreactive reference test results. Also includes 12 IDS without retest results.

Overall Clinical Specificity of the PROCLEIX® ULTRIO® Assay

There were 12,028 pools tested with the PROCLEIX® ULTRIO® Assay and included in the specificity calculations (Table 5).There were 11,796 pools from whole blood donations that tested nonreactive in the PROCLEIX ULTRIO (Table 7). Of these, 11,786 pools were considered true negative and 10 pools were considered false negative. Nine of the 10 false negative pools were HBsAg seropositive and 1 pool was reactive in the PROCLEIX® HIV-1/HCV Assay. There were 232 pools that tested reactive in the PROCLEIX ULTRIO Assay. Of these, 173 pools were considered true positive. Six of these pools were reactive in the PROCLEIX ULTRIO Assay and had a constituent sample that was reactive in Alternate NAT, but were nonreactive in the reference test. Fifty-nine pools were considered false positive. The overall specificity of 16-sample pools from whole blood donations was 11,786/11,845 or 99.5% (95% CI: 99.4-99.6%).

There were 12,780 IDS tested with the PROCLEIX ULTRIO Assay – from reactive pools or tested as IDS only – and included in the specificity calculations (Table 6).There were 12,480 IDS that tested nonreactive in the PROCLEIX ULTRIO Assay (Table 7). Of these, 12,479 IDS were considered true negative and 1 sample was considered false negative. The false negative sample was PROCLEIX HIV-1/HCV Assay reactive, HCV discriminated. There were 300 IDS that tested reactive in the PROCLEIX ULTRIO Assay. Of these, 193 IDS were considered true positive and 107 IDS were considered false positive. The overall specificity of IDS from whole blood donations was 12,479/12,586 or 99.1% (95% CI: 99.0-99.3%).

The specificity of the PROCLEIX ULTRIO Assay was also calculated based on the total number of specimens tested either as IDS or in a pool. This included 216,180 donor samples tested either as IDS, IDS after a reactive pool result, or as part of a non-reactive pool (all 16 samples from a non-reactive pool are considered non-reactive). All donor samples tested had valid PROCLEIX ULTRIO Assay results, PROCLEIX HIV-1/HCV Assay (including the appropriate discriminatory assay) results and HBsAg serology results. After complete resolution, eleven samples were considered false negative and 193 were considered true positive. Of the remaining 215,976 samples, 107 were considered false positive and 215,869 were considered true negative. The overall specificity from all of the donor samples from whole blood donations was 215,869/215,976 or 99.95% (95% CI: 99.94-99.96%).

There were 1,198 PSP pools tested with the PROCLEIX ULTRIO Assay and included in the specificity calculations (Table 7). There were 1,195 PSP pools that tested nonreactive in the PROCLEIX ULTRIO Assay and all of these pools were considered true negative. There were three PSP pools that tested reactive in the PROCLEIX ULTRIO Assay and all three pools were false positive. The overall specificity of pools from PSP donations was 1,195/1,198 or 99.7% (95% CI: 99.3-99.9%).

Overall Clinical Specificity of the PROCLEIX® HIV-1, HCV, and HBV Discriminatory Assays

There were 1,785 IDS that tested nonreactive in the PROCLEIX® HIV-1 Discriminatory Assay and all were considered true negative (Table 7). There were 12 IDS that tested reactive in the PROCLEIX HIV-1 Discriminatory Assay. Of these, 8 IDS were considered true positive and 4 IDS were considered false positive. The specificity of the PROCLEIX HIV-1 Discriminatory Assay was 99.8% (1,785/1,789; 95% CI: 99.4-99.9%).

There were 1,653 IDS that tested nonreactive in the PROCLEIX® HCV Discriminatory Assay and all were considered true negative. There were 157 IDS that tested reactive in the PROCLEIX HCV Discriminatory Assay. Of these, 125 IDS were considered true positive and 32 IDS were considered false positive. The specificity of the PROCLEIX HCV Discriminatory Assay was 98.1% (1,653/1,685; 95% CI: 97.3-98.7%).

There were 1,748 IDS that tested nonreactive in the PROCLEIX® HBV Discriminatory Assay and all were considered true negative. There were 47 IDS that tested reactive in the PROCLEIX HBV Discriminatory Assay. Of these, 43 IDS were considered true positive and 4 IDS were considered false positive. The specificity of the PROCLEIX HBV Discriminatory Assay was 99.8% (1,748/1,752; 95% CI: 99.4-99.9%).

Table 7. PROCLEIX® System - Clinical Specificity Study: Overall Specificities of the PROCLEIX® ULTRIO® Assay and Discriminatory Assays

Assay	Sample	N	True Negative	False Negative	True Positive	False Positive	Specificity (%)	95% CI
PROCLEIX® ULTRIO® Assay	Pools* from Whole Blood Donations	12,028	11,786	10	173	59	99.5	99.4-99.6
	IDS** from Whole Blood Donations	12,780	12,479	1	193	107	99.1	99.0-99.3
	IDS and Nonreactive Pools	216,180	215,869	11	193	107	99.95	99.94-99.96
	Pools from Paid Source Plasma Donations	1,198	1,195	0	0	3	99.7	99.3-99.9
PROCLEIX® HIV-1 Discriminatory Assay	IDS from Whole Blood Donations	1,797	1,785	0	8	4	99.8	99.4-99.9
PROCLEIX® HCV Discriminatory Assay	IDS from Whole Blood Donations	1,810	1,653	0	125	32	98.1	97.3-98.7
PROCLEIX® HBV Discriminatory Assay	IDS from Whole Blood Donations	1,795	1,748	0	43	4	99.8	99.4-99.9

N = Number of samples (individual donations or pools)
CI = Confidence Interval
*Pools included 303 donor samples from volunteer source plasma donations
**IDS included 9 donor samples from volunteer source plasma donations.

Clinical Specificity of the PROCLEIX® ULTRIO® Assay and Discriminatory Assays by Site

Table 8 shows clinical specificity results for the three blood center testing sites. Clinical specificity of the PROCLEIX® ULTRIO® Assay in 16-sample pools ranged from 99.3% (95% CI: 99.1-99.5%) for Site 2 to 99.8% (95% CI: 99.6-99.9%) for Site 1. Specificity in IDS was significantly lower at Site 2 at 98.5% (95% CI: 98.1-98.8%) than at Sites 1 and 3, which had specificity rates of 99.6% (95% CI: 99.3-99.8%) and 99.5% (95% CI: 99.2-99.7%), respectively. Including all samples tested, whether tested as IDS only, IDS after a reactive pool result, or as part of a nonreactive pool, specificity ranged from 99.930% (95% CI: 99.912-99.945%) for Site 2 to 99.973% (95% CI: 99.955-99.984%) for Site 1.

Clinical specificity of the PROCLEIX® HIV-1 Discriminatory Assay ranged from 99.7% (95% CI: 98.7-100%) for Site 3 to 100% (95% CI: 99.3-100%) for Site 1. Specificity of the PROCLEIX® HCV Discriminatory Assay was significantly lower at Site 2 at 96.1% (95% CI: 94.3-97.5%) than at Sites 1 and 3, which had specificity rates of 99.2% (95% CI: 98.1-99.8%) and 99.3% (95% CI: 98.1-99.8%), respectively. For the PROCLEIX® HBV Discriminatory Assay, specificity ranged from 99.6% (95% CI: 98.7-100%) for Site 3 to 100% (95% CI: 99.3-100%) for Site 1. (Table 8)

Table 8. PROCLEIX® System – Clinical Specificity Study: Specificities of the PROCLEIX® ULTRIO® Assay and Discriminatory Assays by Site

Assay	Sample	Site	N	True Negative	False Negative	True Positive	False Positive	Specificity (%)	95% CI
PROCLEIX® ULTRIO® Assay	Pools from Whole Blood Donations	1*	3421	3399	2	13	7	99.8	99.6-99.9
		2	5278	5122	8	114	34	99.3	99.1-99.5
		3	3329	3265	0	46	18	99.5	99.1-99.7
	IDS from Whole Blood Donations	1**	3869	3838	0	15	16	99.6	99.3-99.8
		2	4762	4560	1	130	71	98.5	98.1-98.8
		3	4149	4081	0	48	20	99.5	99.2-99.7
	IDS and Nonreactive Pools	1	58,285	58,252	2	15	16	99.97	99.96-99.98
		2	101,492	101,282	9	130	71	99.93	99.91-99.95
		3	56,403	56,335	0	48	20	99.97	99.95-99.98
PROCLEIX® HIV-1 Discriminatory Assay	IDS from Whole Blood Donations	1	532	532	0	0	0	100	99.3-100
		2	690	682	0	6	2	99.7	98.9-100
		3	575	571	0	2	2	99.7	98.7-100
PROCLEIX® HCV Discriminatory Assay	IDS from Whole Blood Donations	1	535	522	0	9	4	99.2	98.1-99.8
		2	698	592	0	82	24	96.1	94.3-97.5
		3	577	539	0	34	4	99.3	98.1-99.8
PROCLEIX® HBV Discriminatory Assay	IDS from Whole Blood Donations	1	535	530	0	5	0	100	99.3-100
		2	706	674	0	30	2	99.7	98.9-100
		3	554	544	0	8	2	99.6	98.7-100

N = number of samples (individual donations or pools)
CI = Confidence Interval
* Pools included 303 donor samples from volunteer source plasma donations.
** IDS included nine donor samples from volunteer source plasma donations.

False Positive Rates of the PROCLEIX® ULTRIO® Assay in Pools and IDS from Whole Blood Donations

False positive rates for the PROCLEIX® ULTRIO® Assay are shown in Table 9a and Table 9b for pools and IDS, respectively. For the PROCLEIX ULTRIO Assay clinical trial, pools and IDS were considered false positive if samples were PROCLEIX ULTRIO Assay reactive, reference test (PROCLEIX® HIV-1/HCV Assay and HBsAg test) nonreactive, and Alternate NAT nonreactive or not tested.

Table 9a. Clinical Specificity Study: PROCLEIX® ULTRIO® Assay False Positive Rates in 16-Sample Pools

Results	False Positive Rates
Multiplex testing of pools	0.50% (59/11,845)
Pools with 16 multiplex nonreactive IDS	0.42% (50/11,845)
Pools with at least 1 multiplex reactive, discriminatory reactive IDS	0.02% (2/11,845)
Pools with at least 1 multiplex reactive, discriminatory nonreactive IDS	0.06% (7/11,845)

Table 9b. Clinical Specificity Study: PROCLEIX® ULTRIO® Assay False Positive Rates in IDS

Results	False Positive Rates
Multiplex testing of IDS	0.85% (107/12,586)
Multiplex reactive, discriminatory reactive IDS	0.08% (10/12,586)
Multiplex reactive, discriminatory nonreactive IDS	0.77% (97/12,586)*

*Six of the 97 nondiscriminated IDS were not tested in all discriminatory assays

Comparison of the PROCLEIX® ULTRIO® Assay to the PROCLEIX® HIV-1/HCV Assay

Table 10 shows the reactivity rates of the PROCLEIX® ULTRIO® Assay and PROCLEIX® HIV-1/HCV Assay in HIV-1 and HCV positive donations collected during the clinical specificity study for the PROCLEIX ULTRIO Assay. Samples (whether tested in pools or as IDS only) were included in this analysis if they had valid and complete PROCLEIX ULTRIO Assay and PROCLEIX HIV-1/HCV Assay results for HIV-1 and HCV detection.

All of the eight HIV-1 positive samples were detected with both the PROCLEIX ULTRIO Assay and the PROCLEIX HIV-1/HCV Assay. Of 127 HCV positive samples, 125 samples (98.4%) were detected with the PROCLEIX ULTRIO Assay. Two of the 125 samples were reactive for HCV with the PROCLEIX ULTRIO Assay and HCV Alternate NAT, but were nonreactive with the PROCLEIX HIV-1/HCV Assay. Likewise, 125 of 127 HCV positive samples (98.4%) were detected with the PROCLEIX HIV-1/HCV Assay. Two of the 125 samples were reactive for HCV with the PROCLEIX HIV-1/HCV Assay but nonreactive with the PROCLEIX ULTRIO Assay.

The results demonstrate that the PROCLEIX ULTRIO Assay and PROCLEIX HIV-1/HCV Assay detected HIV-1 and HCV equally. Both assays detected the same number of positive samples. Therefore, sensitivity of the PROCLEIX ULTRIO Assay is similar to that of the PROCLEIX HIV-1/HCV Assay.

Table 10. Comparison of Reactivity Rates between the PROCLEIX® ULTRIO® Assay and the PROCLEIX® HIV-1/HCV Assay in HIV-1 and HCV Infected Donations

Target	RNA Positive Donations*	Reactivity Rate	
		PROCLEIX® ULTRIO® Assay	PROCLEIX® HIV-1/HCV Assay
HIV-1	8	100% (8/8)	100% (8/8)
HCV	127	98.4% (125/127)**	98.4% (125/127)***

* Number of positive donor samples reactive by the PROCLEIX HIV-1/HCV Assay and/or by the PROCLEIX ULTRIO Assay and Alternate NAT.
** Two of the 125 positive samples were reactive by Alternate NAT but nonreactive by the PROCLEIX HIV-1/HCV Assay. One of the two samples was also HCV seropositive.
*** Two of the 125 positive donor samples were reactive with the PROCLEIX HIV-1/HCV Assay only.

Comparison of the PROCLEIX® ULTRIO® Assay to HIV-1 and HCV Serology Results

Results generated from the pooled, individual donation and discriminatory assay specificity studies allow comparison of the PROCLEIX® ULTRIO® Assay with serology reactivity (Table 11). All of the specimens included in this analysis were EIA repeat reactive.

HIV-1 Western Blot results were available for 67 samples. Of these, two were HIV-1 Western Blot positive: both samples were PROCLEIX ULTRIO Assay reactive (100%). The remaining 65 of 67 had negative or indeterminate Western Blot results: all 65 (100%) were PROCLEIX ULTRIO Assay nonreactive.

HCV RIBA® Assay results were available for 180 samples. Of these, 44 were RIBA Assay positive: 37 (84.1%) were PROCLEIX ULTRIO Assay reactive and seven were PROCLEIX ULTRIO Assay nonreactive. The remaining 136 of 180 had RIBA Assay negative or indeterminate results: all 136 (100%) were PROCLEIX ULTRIO Assay nonreactive.

Table 11. Clinical Specificity Study: Comparison of HIV-1 and HCV Confirmatory Serology and PROCLEIX® ULTRIO® Assay Results

Serology			PROCLEIX® ULTRIO® Assay*	
HIV-1 EIA RR (n=75)	Western Blot Result		Reactive	Non-reactive
	Positive	2	2	0
	Indeterminate	44	0	44
	Negative	21	0	21
	Not available	8	0	8
HCV EIA RR (n=197)	RIBA Assay Results			
	Positive	44	37	7**
	Indeterminate	51	0	51
	Negative	85	0	85
	Not available	17	6	11

RR: Repeatedly reactive
* Includes samples with dHIV-1 or dHCV results or with nonreactive pool or IDS-only results.
** Seven samples that were HCV positive by the RIBA Assay came from PROCLEIX ULTRIO Assay nonreactive pools, so discriminatory testing was not required.

Comparison of the PROCLEIX® ULTRIO® Assay to HBV Serology Results

Table 12 summarizes the HBV results for 218,260 samples that were tested initially in pools or as IDS only and had valid PROCLEIX® ULTRIO® Assay and PROCLEIX® HBV Discriminatory Assay results.

Of 218,260 donor samples tested in the clinical specificity study in the PROCLEIX ULTRIO Assay and HBV Discriminatory Assay, 216,949 (99.40%) were PROCLEIX ULTRIO Assay nonreactive and HBsAg and anti-HBc seronegative, indicating no evidence of previous HBV exposure. One of the 216,949 was PROCLEIX ULTRIO Assay nonreactive, HBsAg and anti-HBc serology negative, and HBV Discriminatory Assay reactive. Of the remaining 1,311 of 218,260 specimens, 46 samples were reactive for HBV DNA in the PROCLEIX ULTRIO Assay and HBV Discriminatory Assay and 1,265 were nonreactive.

Thirty-eight of 46 IDS samples were HBsAg seropositive (i.e., true positive) and 8 (of the 46) were HBsAg seronegative. Three of these 8 specimens were categorized as false positive as HBV was not detected by Alternate NAT, or Alternate NAT results were unavailable due to insufficient sample volume. Of these three, one was PROCLEIX ULTRIO Assay nonreactive and seronegative at follow-up, 2.5 weeks after the index donation. Alternate NAT detected HBV in the remaining 5 (of 8) specimens. Two of these 5 cases were followed-up 3 to 5.5 weeks after the index donation and were both nonreactive in the PROCLEIX ULTRIO Assay and HBV Alternate NAT. HBsAg and anti-HBc results were also seronegative at follow-up.

Of the 1,265 samples that showed evidence of HBV exposure by serology but were nonreactive for HBV DNA, 1,254 were anti-HBc seropositive only samples. The sample pattern of HBV DNA and HBsAg non-reactivity and anti-HBc reactivity would be observed in fully resolved HBV infections as well as in samples with false positive anti-HBc serologic test results. While resolved infections may be present in this population, a significant proportion of these results may be due to false positive anti-HBc results.

Among the remaining 11 HBV seropositive, HBV DNA negative samples, seven were HBsAg seropositive and anti-HBc seroreactive (or seropositive) and four were only HBsAg seropositive. Of these four samples, 2 samples were not detected in Alternate NAT, showing consistency with PROCLEIX ULTRIO Assay results (i.e., true negative results). Alternate NAT results for the remaining samples are unavailable.

Table 12. PROCLEIX® System - Clinical Specificity Study: Comparison of HBV Serology and PROCLEIX® ULTRIO® Assay Results at Index

Line	Initial Results	N	%
1	Anti-HBc + / HBsAg + / DNA +	38	0.017
2	Anti-HBc + / HBsAg + / DNA -	7	0.003
3	Anti-HBc - / HBsAg + / DNA +	0	0.000
4	Anti-HBc - / HBsAg + / DNA -	4	0.002
5	Anti-HBc - / HBsAg - / DNA +	7*	0.003
6	Anti-HBc - / HBsAg - / DNA -	216,949	99.399
7	Anti-HBc + / HBsAg - / DNA +	1	0.001
8	Anti-HBc + / HBsAg - / DNA -	1,254	0.575
Total		218,260	

N = number of samples
Anti-HBc + = seropositive for HBV core antibody
HBsAg + = seropositive for HBsAg
DNA + = PROCLEIX ULTRIO Assay reactive, HBV discriminated
Anti-HBc - = seronegative for HBV core antibody
HBsAg - = seronegative for HBsAg
DNA - = nonreactive in the dHBV Assay or PROCLEIX ULTRIO Assay nonreactive, dHBV Assay reactive
*Three of 7 donors were followed up. All of the follow-up specimens were PROCLEIX ULTRIO Assay nonreactive and HBsAg seronegative

NON-SPECIFICITY STUDIES

PROCLEIX® SYSTEM

Specificity and Sensitivity of the PROCLEIX® ULTRIO® Assay and Discriminatory Assays in the Presence of Donor and Donation Factors

Tables 13 and 14 show all valid initial test results obtained when specimens containing various donor and donation factors were tested with the PROCLEIX® ULTRIO® Assay and Discriminatory Assays. HIV-1, HCV, and HBV positive specimens were created by individually spiking the various donor and donation specimens to a final concentration of 200 copies/mL of HIV-1, 60 IU/mL of HCV, or 30 IU/mL of HBV. Cross-reactivity and interference are defined as greater than 5% unexpected results.

No cross-reactivity (Table 14) or interference (Table 13) was observed for naturally occurring hemolyzed or lipemic specimens or plasma containing the following substances: serum albumin (6 g/dL), hemoglobin (500 mg/dL) and lipids (3,000 mg/dL). No cross-reactivity or interference for detection of HIV-1, HCV, and HBV was observed for naturally occurring icteric specimens or plasma containing bilirubin up to 20 mg/dL. However, this high level of spiked bilirubin produces a slight decrease in HBV analytical sensitivity. This effect was not observed when bilirubin is present at 2.5 mg/dL.

Multiple specimens from each group of patients with the following autoimmune conditions were evaluated: rheumatoid factor, antinuclear antibody, lupus and multiple myeloma. Also tested were samples from flu vaccinees, from hepatitis B vaccinees, from patients with elevated IgM, with elevated IgG, with elevated amino alanine transferase (ALT) and from patients with alcoholic liver cirrhosis. For the majority of these conditions, no cross-reactivity or interference was observed. However, a small portion of these specimens had random, unexpected results in greater than 5% of the samples tested. These occurrences are indicated in bold text in Tables 13 and 14.

No cross-reactivity or interference was observed in the majority of bacterially contaminated plasma specimens or in specimens from patients infected with other bloodborne pathogens. Multiple specimens from each group of patients with the following viral infections were evaluated: herpes simplex virus-1 (HSV 1), herpes simplex virus-2 (HSV 2), CMV, EBV, hepatitis A virus (HAV), HTLV-I, HTLV-II, hepatitis G virus (HGV), rubella, and parvovirus B-19. A small portion of the specimens containing viral infections had random, unexpected results in greater than 5% of the samples tested. These occurrences are indicated in bold text in Tables 13 and 14.

Table 13. Detection of HIV-1, HCV, and HBV in the Presence of Donor and Donation Factors with the PROCLEIX® ULTRIO® Assay and Discriminatory Assays

Donor or Donation Factor	Reactive/Tested*					
	HIV-1 Positive (200 c/mL)		HCV Positive (60 IU/mL)		HBV Positive (30 IU/mL)	
	PROCLEIX® ULTRIO® Assay	dHIV-1	PROCLEIX® ULTRIO® Assay	dHCV	PROCLEIX® ULTRIO® Assay	dHBV
Hemolyzed	21/21	21/21	18/18	18/18	29/30	30/30
Icteric	21/21	21/21	30/30	30/30	30/30	30/30
Lipemic	24/24	24/24	12/12	12/12	30/30	29/30
Normal	39/39	37/37	39/39	39/39	39/39	39/39
Albumin (6 g/dL)	39/39	39/39	39/39	39/39	38/39	39/39
Bilirubin (20 mg/dL)	39/39	39/39	39/39	39/39	38/39	**37/39****
Bilirubin (2.5 mg/dL)	NA	NA	NA	NA	86/87	89/89
Hemoglobin (500 mg/dL)	38/38	39/39	39/39	39/39	39/39	39/39
Lipids (3000 mg/dL)	39/39	39/39	39/39	39/39	38/39	39/39
Alcoholic Cirrhosis	30/30	30/30	30/30	**28/30**	30/30	29/30
Antinuclear Antibody	27/27	27/27	27/27	26/27	27/27	27/27
ALT	30/30	30/30	30/30	30/30	30/30	30/30
Elevated IgG	30/30	**26/30**	**25/30**	30/30	30/30	30/30
Elevated IgM	29/30	**27/30**	29/30	29/30	**26/30**	**27/30**
Lupus	**28/30**	30/30	30/30	30/30	30/30	30/30
Multiple Myeloma	23/23	23/23	23/23	23/23	**21/23**	23/23
Rheumatoid Factor	**27/30**	29/30	29/30	30/30	29/30	30/30
Flu Vaccinee	30/30	30/30	30/30	30/30	30/30	30/30
HBV Vaccinee	30/30	30/30	30/30	30/30	29/30	29/30
C albicans	30/30	30/30	30/30	30/30	30/30	30/30
C diphtheriae	30/30	30/30	30/30	30/30	31/31	31/31
M luteus	30/30	30/30	30/30	30/30	29/30	30/30
P acnes	30/30	30/30	30/30	30/30	29/30	29/30
P carinii	30/30	30/30	30/30	30/30	30/30	30/30
S aureus	30/30	30/30	30/30	30/30	30/30	30/30
S epidermidis	30/30	30/30	30/30	30/30	28/29	29/29
CMV	30/30	30/30	30/30	30/30	**28/30**	30/30
EBV	30/30	30/30	30/30	30/30	30/30	30/30
HAV	30/30	30/30	30/30	29/30	**26/30**	**28/30**
HSV 2	30/30	30/30	30/30	30/30	29/30	30/30
HSV 1	30/30	30/30	30/30	30/30	30/30	30/30
HTLV II	30/30	30/30	**27/30**	**27/30**	**26/30**	28/29
Rubella	30/30	30/30	30/30	30/30	30/30	29/30
HGV	21/21	21/21	21/21	**18/21**	20/21	21/21
Parvovirus B19	30/30	30/30	30/30	30/30	**27/30**	**27/30**
HTLV I	30/30	30/30	30/30	30/30	29/30	**28/30**
Controls	270/270	269/270	270/270	270/270	259/270	263/270

NA = Not tested
* Combined results from three clinical lots of reagents.
** Bolded text indicates greater than 5% nonreactive results.

Table 14. Specificity of the PROCLEIX® ULTRIO® Assay and Discriminatory Assays in the Presence of Donor and Donation Factors

Donor or Donation Factor	Nonreactive/Negative Samples Tested*			
	PROCLEIX® ULTRIO® Assay	dHIV-1	dHCV	dHBV
Hemolyzed	30/30	30/30	30/30	30/30
Icteric	29/30	30/30	29/30	29/30
Lipemic	30/30	30/30	29/30	30/30
Normal	36/36	36/36	36/36	36/36
Albumin (6 g/dL)	36/36	36/36	36/36	36/36
Bilirubin (20 mg/dL)	36/36	36/36	36/36	36/36
Hemoglobin (500 mg/dL)	36/36	35/35	36/36	36/36
Lipids (3000 mg/dL)	36/36	35/35	36/36	36/36
Alcoholic Cirrhosis	30/30	30/30	30/30	30/30
Antinuclear Antibody	**24/27****	27/27	26/27	**19/27**
ALT	29/30	30/30	30/30	30/30
Elevated IgG	**27/30**	30/30	30/30	30/30
Elevated IgM	30/30	30/30	30/30	30/30
Lupus	29/30	30/30	30/30	29/30
Multiple Myeloma	**21/24**	24/24	24/24	**22/24**
Rheumatoid Factor	30/30	30/30	30/30	29/30
Flu Vaccinee	**27/30**	30/30	30/30	30/30
HBV Vaccinee	30/30	30/30	29/30	30/30
C albicans	30/30	30/30	30/30	30/30
C diphtheriae	30/30	30/30	30/30	29/30
M luteus	29/30	30/30	30/30	30/30
P acnes	30/30	30/30	29/30	30/30
P carinii	30/30	30/30	29/30	30/30
S aureus	30/30	30/30	**28/30**	30/30
S epidermidis	30/30	30/30	30/30	30/30
CMV	**28/30**	30/30	30/30	**27/30**
EBV	**30/33**	33/33	33/33	**29/33**
HAV	30/30	30/30	30/30	29/30
HSV 2	26/27	30/30	**28/30**	**27/30**
HSV 1	**26/30**	27/27	27/27	**25/27**
HTLV II	30/30	30/30	29/30	30/30
Rubella	29/30	30/30	30/30	30/30
HGV	15/15	15/15	15/15	15/15
Parvovirus B19	**24/27**	27/27	27/27	27/27
HTLV I	27/27	27/27	27/27	27/27
Controls	269/270	270/270	269/270	270/270

* Combined results from three clinical lots of reagents.
** Bolded text indicates greater than 5% reactive results.

Specificity and Sensitivity of the PROCLEIX® ULTRIO® Assay and Discriminatory Assays in the Presence of Anticoagulants and Serum

The sensitivity and specificity of the PROCLEIX® ULTRIO® Assay and Discriminatory Assays for serum samples and samples collected in various anticoagulants is shown in Tables 15 and 16. Detection rates were calculated from valid initial results. Cross-reactivity and interference are defined as greater than 5% unexpected results. The anticoagulants tested were ACD (Acid Citrate Dextrose), K_2 EDTA (ethylene diamine tetraacetic acid), K_3 EDTA, PPT (K_2 EDTA Plasma Preparation Tube), sodium citrate, CPD (citrate phosphate dextrose), and sodium heparin. For the majority of anticoagulants, no cross-reactivity or interference for detection of HIV-1, HCV, or HBV was observed. A small portion of the anticoagulants tested had random, unexpected results in greater than 5% of the samples tested. These occurrences are indicated in bold text in Tables 15 and 16.

Table 15. Detection of HIV-1, HCV and HBV in the Presence of Anticoagulants and Serum with the PROCLEIX® ULTRIO® Assay and Discriminatory Assays

Anticoagulant	Reactive/Tested (Percent Reactive)					
	HIV-1 Positive* (200 c/mL)		HCV Positive* (60 IU/mL)		HBV Positive** (up to 30 IU/mL)	
	PROCLEIX® ULTRIO® Assay	dHIV-1	PROCLEIX® ULTRIO® Assay	dHCV	PROCLEIX® ULTRIO® Assay	dHBV
ACD	30/30 (100%)	30/30 (100%)	29/30 (96.7%)	30/30 (100%)	**122/130 (93.8%)*** **	125/128 (97.7%)
CPD	29/30 (96.7%)	30/30 (100%)	29/30 (96.7%)	29/30 (96.7%)	124/129 (96.1%)	**122/129 (94.6%)**
K_2 EDTA	30/30 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)	127/130 (97.7%)	129/130 (99.2%)
K_3 EDTA	29/30 (96.7%)	30/30 (100%)	30/30 (100%)	30/30 (100%)	125/130 (96.2%)	125/130 (96.2%)
Sodium Citrate	29/30 (96.7%)	30/30 (100%)	30/30 (100%)	30/30 (100%)	**122/130 (93.8%)**	126/130 (96.9%)
Sodium Heparin	30/30 (100%)	30/30 (100%)	29/30 (96.7%)	30/30 (100%)	**121/129 (93.8%)**	126/129 (97.7%)
PPT	30/30 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)	121/126 (96.0%)	**119/126 (94.4%)**
Serum	30/30 (100%)	30/30 (100%)	29/30 (96.7%)	30/30 (100%)	**123/130 (94.6%)**	127/130 (97.7%)

* Combined results from three clinical lots of reagents.
** Combined results from five clinical lots of reagents for ACD, CPD, sodium citrate, sodium heparin, PPT, and serum. Results for K_2 EDTA and K_3 EDTA were from four clinical lots.
*** Bolded text indicates greater than 5% nonreactive results.

Table 16. Specificity of the PROCLEIX® ULTRIO® Assay and Discriminatory Assays in the Presence of Anticoagulants and Serum

Anticoagulant	Nonreactive/Negative Samples Tested* (Percent Nonreactive)			
	PROCLEIX® ULTRIO® Assay	dHIV-1	dHCV	dHBV
ACD	30/30 (100%)	30/30 (100%)	29/30 (96.7%)	29/30 (96.7%)
CPD	**28/30 (93.3%)****	30/30 (100%)	30/30 (100%)	30/30 (100%)
K2 EDTA	30/30 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)
K3 EDTA	30/30 (100%)	30/30 (100%)	30/30 (100%)	29/30 (96.7%)
Sodium Citrate	30/30 (100%)	30/30 (100%)	30/30 (100%)	29/30 (96.7%)
Sodium Heparin	30/30 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)
PPT	30/30 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)
Serum	29/30 (96.7%)	30/30 (100%)	30/30 (100%)	30/30 (100%)

* Combined results from three clinical lots of reagents.
** Bolded text indicates greater than 5% reactive results.

CLINICAL SENSITIVITY

PROCLEIX® SYSTEM

Testing of Known Positive Samples from HIV-1, HCV, and/or HBV Infected Individuals

A combined total of 3,138 specimens known to be positive for HIV-1 RNA, or HCV RNA, or HBV DNA were procured from a vendor and were included in the clinical sensitivity analyses.

These specimens were classified as HIV-1 RNA positives, HCV RNA positives, and HBV DNA positives based on qualitative nucleic acid testing (NAT) results. In addition to NAT, the vendor provided serologic test results to confirm that samples were positive for the appropriate target and that samples were not co-infected. HIV-1 positive samples were seroreactive for HIV-1 antibody and negative for HCV antibody and HBsAg. Likewise, HCV positive samples were serologically reactive for HCV antibody and negative for HIV-1 antibody and HBsAg. All but two of the HBV positive samples were positive for HBsAg and negative for HIV-1 and HCV antibody. Two HBV positive samples included in the clinical sensitivity calculations for the PROCLEIX® ULTRIO® Assay and PROCLEIX® HBV Discriminatory Assay were negative for HBsAg but were positive for HBV core antibody (and negative for HIV-1 and HCV antibody).

The clinical sensitivity study was performed at three testing sites using three Clinical Lots of the PROCLEIX ULTRIO Assay. The positive samples were tested undiluted (neat) with the PROCLEIX ULTRIO Assay, PROCLEIX® HIV-1 Discriminatory Assay (dHIV-1), PROCLEIX® HCV Discriminatory Assay (dHCV), and PROCLEIX HBV Discriminatory Assay (dHBV) and tested diluted (1:16) with the PROCLEIX ULTRIO Assay. All dilutions were made with serum known to be negative for HIV-1 antibody and RNA, HCV antibody and RNA, and HBsAg and HBV DNA. In addition, negative serum samples were tested with the PROCLEIX ULTRIO Assay and three discriminatory assays at each clinical site as a control for potential study bias.

Known-positive samples with nonreactive (discordant) results were tested neat with quantitative Alternative NAT, along with some known-positive samples with reactive (concordant) results to control for bias. Known-positive samples with viral loads less than the Alternate NAT's quantitative limit of detection (LOD) when tested neat were excluded from the clinical sensitivity analyses, regardless of whether the PROCLEIX ULTRIO Assay results were discordant or concordant. Because the LOD of the Alternate NAT is the same or similar to the PROCLEIX ULTRIO Assay sensitivity claim, the viral loads of these samples were considered below or potentially below the PROCLEIX ULTRIO Assay sensitivity claim. Therefore, the sensitivities presented below include samples with known HIV-1 RNA, HCV RNA, and HBV DNA concentrations at or above the PROCLEIX ULTRIO Assay sensitivity claim when tested neat (results were not corrected for dilution). Also included are samples with unknown viral concentration; not all samples had viral load quantitation performed.

The sensitivity for the PROCLEIX ULTRIO Assay and PROCLEIX HIV-1 Discriminatory Assay for undiluted (neat) HIV-1 positive samples was 100% (95% CI: 99.7-100%) and 99.9% (95% CI: 99.5-100%), respectively (Table 17). The sensitivity for the PROCLEIX ULTRIO Assay for diluted (1:16) HIV-1 positive samples was 99.0% (95% CI: 98.2-99.5%).

The sensitivity for the PROCLEIX ULTRIO Assay and the PROCLEIX HCV Discriminatory Assay for undiluted (neat) HCV positive samples was 99.7% (95% CI: 99.1-99.9%) and 99.9% (95% CI: 99.5-100%), respectively. The sensitivity for the PROCLEIX ULTRIO Assay for diluted (1:16) HCV positive samples was 99.3% (95% CI: 98.6-99.7%).

The sensitivity for the PROCLEIX ULTRIO Assay and PROCLEIX HBV Discriminatory Assay for undiluted (neat) HBV positive samples was 98.9% (95% CI: 98.1-99.5%) and 99.3% (95% CI: 98.6-99.7%), respectively. The sensitivity for the PROCLEIX ULTRIO Assay for diluted (1:16) HBV positive samples was 90.5% (95% CI: 88.5-92.2%).

The overall clinical sensitivity for the PROCLEIX ULTRIO Assay, which takes into account all positive samples tested, was 99.6% (95% CI: 99.3-99.8%) for undiluted (neat) positive samples and 96.3% (95% CI: 95.6-96.9%) for diluted (1:16) positive samples.

Table 17. PROCLEIX® System - Sensitivity of the PROCLEIX® ULTRIO® Assay and Discriminatory Assays in Known Positive Samples

Assay	Sample	N	Reactive	Sensitivity (%)	95% CI
PROCLEIX® ULTRIO® Assay (Neat)	All	3,136	3,122	99.6	99.3-99.8
	HIV-1 Only	1,076	1,076	100	99.7-100
	HCV Only	1,028	1,025	99.7	99.1-99.9
	HBV Only	1,032	1,021	98.9	98.1-99.5
PROCLEIX® ULTRIO® Assay (Diluted 1:16)	All	3,138	3,022	96.3	95.6-96.9
	HIV-1 Only	1,077	1,066	99.0	98.2-99.5
	HCV Only	1,029	1,022	99.3	98.6-99.7
	HBV Only	1,032	934	90.5	88.5-92.2
dHIV-1	HIV-1 Only	1,076	1,075	99.9	99.5-100
dHCV	HCV Only	1,029	1,028	99.9	99.5-100
dHBV	HBV Only	1,028	1,021	99.3	98.6-99.7

N = number of samples
CI = Confidence Interval

Testing of Known Positive 16-Sample Pools

The clinical sensitivity of the PROCLEIX® ULTRIO® Assay was evaluated in 190 sixteen-member pools composed of 1 to 3 HIV-1, HCV, and/or HBV known positive samples and 13 to 15 negative samples. All the positive samples were collected throughout the United States. Their viral positivity was identified by commercial HIV-1 RNA and HCV RNA assays and a validated HBV DNA assay. Two clinical sites participated in the study using one Clinical Lot of the PROCLEIX ULTRIO Assay. Known-negative pools were tested with the PROCLEIX ULTRIO Assay at each clinical site as a control for potential study bias.

Known-positive samples from pools with nonreactive results were tested neat with quantitative Alternate NAT, along with some known-positive samples from pools with reactive results to control for bias. Known-positive pools with viral loads below the PROCLEIX ULTRIO Assay sensitivity claim were excluded from the clinical sensitivity analyses. Therefore, the sensitivities presented in Table 18 include pools with confirmed viral loads at or above the PROCLEIX ULTRIO Assay sensitivity claim or of unknown viral concentration; not all pools had viral load quantitation performed.

Overall, the sensitivity for the PROCLEIX ULTRIO Assay for 190 known-positive pools containing HIV-1 RNA, HCV RNA, and/or HBV DNA was 97.9% (95% CI: 94.7-99.4%) (Table 18). The sensitivity for the PROCLEIX ULTRIO Assay for 125 HIV-1 known-positive pools was 100% (95% CI: 97.1-100%). The sensitivity for the PROCLEIX ULTRIO Assay for 115 HCV known-positive pools was 100% (95% CI: 96.8-100%). The sensitivity for the PROCLEIX ULTRIO Assay for 123 HBV known-positive pools was 96.7% (95% CI: 91.9-99.1%).

Table 18. PROCLEIX® System - Sensitivity of the PROCLEIX® ULTRIO® Assay in 16-Sample Pools Containing Known Positive Specimens

Pools*	N	Reactive	Sensitivity (%)	95% CI
All**	190	186	97.9	94.7-99.4
HIV-1	125	125	100	97.1-100
HCV	115	115	100	96.8-100
HBV	123***	119	96.7	91.9-99.1

N = number of samples
CI = Confidence Interval
* Pools with confirmed viral loads greater than or equal to the PROCLEIX ULTRIO Assay sensitivity claim or with unknown copy levels.
** All pools containing single analytes or a combination of analytes
*** The neat positive samples from the 4 of 123 pools with PROCLEIX ULTRIO Assay nonreactive results had viral loads of 9,700, 3,800, 6,200 and 9,500 copies/mL at initial quantitation. Viral loads were not determined for all positive samples in the remaining 119 pools.

PROCLEIX® SYSTEM

Clinical Sensitivity High-Risk Population Study

Plasma specimens from individuals at high risk for infection with HIV-1, HCV, and/or HBV were evaluated for the clinical sensitivity high-risk population study. Of the total of 503 high-risk subjects included in the study, the majority reported injection drug use as a risk factor. Other risk factors included multiple sex partners, needle stick accident, blood or blood product transfusion, history of a STD, previous diagnosis of HIV-1, HCV, or HBV infection and dialysis. All the specimens from qualified high-risk subjects were aliquoted and tested undiluted (neat) and diluted (1:16) at one clinical site. The neat specimens were tested with the PROCLEIX® ULTRIO® Assay and HIV-1 Discriminatory, HCV Discriminatory, and HBV Discriminatory Assays. The diluted specimens were tested only with the PROCLEIX ULTRIO Assay. Three clinical lots were used for PROCLEIX ULTRIO Assay and discriminatory assay testing.

True status of the samples was based on their completed laboratory results with the licensed PROCLEIX® HIV-1/HCV Assay and corresponding discriminatory assays and results of HBsAg testing. For the PROCLEIX ULTRIO Assay, clinical sensitivity was determined by comparing results (neat and diluted) with the true status of the samples (Table 19a). For the discriminatory assays, results were compared to the true status in neat specimens with PROCLEIX ULTRIO Assay reactive results. For specimens with discordant results, comparisons were further made to HIV-1, HCV, and HBV Alternate NAT. The Alternate NAT results were used in clinical sensitivity calculations to interpret PROCLEIX ULTRIO Assay results.

Of the 503 high risk specimens tested neat and diluted (1:16 to simulate multiplex testing of pools) in the PROCLEIX ULTRIO Assay, 495 and 502 specimens, respectively, had valid PROCLEIX ULTRIO Assay results and completed laboratory results and were included in the clinical sensitivity calculations (Table 19a). Of the 495 specimens tested neat, 369 were HIV-1, HCV, and/or HBV positive in the reference tests. Of these, all were PROCLEIX ULTRIO Assay reactive. Sensitivity was 100% (95% CI: 99.0 - 100%) in neat specimens for HIV-1, HCV, and HBV detection. Of the 502 specimens tested diluted, 373 were HIV-1, HCV, and/or HBV positive in the reference tests. Of these, 370 were PROCLEIX ULTRIO Assay reactive and 3 were PROCLEIX ULTRIO Assay nonreactive. Sensitivity was 99.2% (95% CI: 97.7 – 99.8%) in diluted specimens for detection of HIV-1, HCV, and HBV.

Of the PROCLEIX ULTRIO Assay neat-reactive specimens, 317 specimens had valid HIV-1 Discriminatory Assay and reference test results (Table 19b). Of these, 158 were HIV-1 positive in the reference tests and all were HIV-1 discriminated. Sensitivity of the HIV-1 Discriminatory Assay was 100% (95% CI: 97.7%-100%) in specimens that tested PROCLEIX ULTRIO Assay neat-reactive.

Of the PROCLEIX ULTRIO Assay neat-reactive specimens, 376 specimens had valid HCV Discriminatory Assay and reference test results (Table 19b). Of these, 299 were HCV positive in the reference tests: 298 were HCV discriminated and 1 was HCV Discriminatory Assay nonreactive. Sensitivity of the HCV Discriminatory Assay was 99.7% (95% CI: 98.2%-100%) in specimens that tested PROCLEIX ULTRIO Assay neat-reactive.

Of the PROCLEIX ULTRIO Assay neat-reactive specimens, 311 specimens tested neat had valid HBV Discriminatory Assay and reference test results (Table 19b). Of these, 25 were HBV positive in the reference tests and all were HBV discriminated. Sensitivity of the HBV Discriminatory Assay was 100% (95% CI: 86.3%-100%) in specimens that tested PROCLEIX ULTRIO Assay neat-reactive.

Several specimens were infected with two or more viruses, based on results of the PROCLEIX HIV-1/HCV and Discriminatory Assays and/or HBsAg serologic tests. Of the 503 subject specimens, 92 were co-infected with HIV-1 and HCV, 9 were co-infected with HIV-1 and HBV, 1 was co-infected with HCV and HBV, and 5 were co-infected with HIV-1, HCV, and HBV. All co-infected specimens tested reactive in the PROCLEIX ULTRIO Assay.

Table 19a. PROCLEIX System – Clinical Sensitivity of the PROCLEIX® ULTRIO® Assay in a High Risk Population

Target	Sample	N	ULTRIO Reactive	Reference Test Positive		Reference Test Negative		Sensitivity (%)	95% CI
				TP*	FN*	TN*	FP*		
All	Neat	495	384	369**	0	111	15	100	99.0-100
	Diluted	502	382	370**	3	117	12	99.2	97.7-99.8

N = number of valid specimens with completed lab results, TP = True positive, FN = False negative, TN = True negative, FP = False positive, CI = Confidence interval
*Interpretations of the PROCLEIX ULTRIO Assay results (for calculating sensitivity) when compared to the reference test results.
**92 were co-infected with HIV-1 and HCV, 9 were co-infected with HIV-1 and HBV, 1 was co-infected with HCV and HBV, and 5 were co-infected with HIV-1, HCV, and HBV.

Table 19b. PROCLEIX System – Clinical Sensitivity of the Discriminatory Assays in PROCLEIX® ULTRIO® Assay Neat-Reactive Specimens From a High Risk Population

Assay	N	Discriminatory Assay Reactive	Reference Test Positive		Reference Test Negative		Sensitivity (%)	95% CI
			TP*	FN*	TN*	FP*		
dHIV-1	317	159	158**	0	158	1	100	97.7-100
dHCV	376	307	298***	1	68	9	99.7	98.2-100
dHBV	311	31	25****	0	280	6	100	86.3-100

*Interpretations of the HIV-1, HCV, or HBV Discriminatory Assay results (for calculating sensitivity) when compared to the reference test results.
**87 were co-infected with HIV-1 and HCV, 9 were co-infected with HIV-1 and HBV, and 5 were co-infected with HIV-1, HCV, and HBV.
***91were co-infected with HIV-1 and HCV, 1 was co-infected with HCV and HBV, and 5 were co-infected with HIV-1, HCV, and HBV.
****9 were co-infected with HIV-1 and HBV and 4 were co-infected with HIV-1, HCV, and HBV.

ANALYTICAL SENSITIVITY

Analytical sensitivity panels comprised of serially diluted HIV-1 type B virus, HIV-1 WHO standard (97/656), HCV WHO standard (96/790), and HBV WHO standard (97/746) were used to evaluate assay sensitivity. The HIV-1 type B virus panel was prepared by serial dilution of an HIV-1 type B tissue culture supernatant, which was value assigned using an in-house HIV-1 quantitative assay, which is calibrated with the VQA Standard, from the Virology Quality Assurance Laboratory, Rush-Presbyterian St. Luke's Medical Center, Rush University, Chicago, IL. Four operators, testing 30 replicates of each copy level, ran a total of 120 replicates of each target level with three clinical lots using the PROCLEIX® System. The S/CO and %CV values are the averages of the values calculated for each clinical lot. The 95% confidence intervals of the positivity rates were based on the exact binomial distribution. Estimations of 50% and 95% detection rates by Probit Analysis are provided.

PROCLEIX® System - Detection of HIV-1 type B virus

HIV-1 type B virus detection with the PROCLEIX® ULTRIO® Assay and HIV-1 Discriminatory Assay (dHIV-1) was 100% at 300 copies/mL, 99% at 100 copies/mL and ≥92% at 30 copies/mL for both assays. Positivity rates at 10 copies/mL were 53% and 57% for the PROCLEIX ULTRIO Assay and dHIV-1 Assay. At 3 copies/mL, the detection rates were 25% and 24% for the PROCLEIX ULTRIO Assay and dHIV-1 Assay. Although there was variability between the two assays, the differences were not statistically significant as indicated by overlapping 95% confidence intervals (Table 20). Detection rates were calculated from valid initial results.

Table 20. PROCLEIX® System - Detection of HIV-1 Type B in Analytical Sensitivity Panels

	PROCLEIX® ULTRIO® Assay						dHIV-1					
HIV-1 B* copies/mL	Number of reactive/ tested**	% Positive	95% Confidence Limits		Average S/CO	%CV	Number of reactive/ tested**	% Positive	95% Confidence Limits		Average S/CO	%CV
			Lower	Upper					Lower	Upper		
300	120/120	100	97	100	15.08	6	120/120	100	97	100	21.44	15
100	119/120	99	95	100	13.34	13	119/120	99	95	100	18.75	26
30	110/120	92	85	96	9.53	31	112/118	95	89	98	12.97	44
10	64/120	53	44	62	6.97	56	68/120	57	47	66	8.80	71
3	30/120	25	18	34	6.91	52	29/120	24	17	33	5.87	114
0	0/120	0	0	3	0.11	87	0/119	0	0	3	0.12	70

*HIV-1 B tissue culture supernatant value assigned with VQA standard.
** Invalid reactions were not included.

PROCLEIX® System - Detection of HIV-1 WHO Standard (97/656)

Detection of the HIV-1 WHO standard with the dHIV-1 Assay was 100% at 600, 200 and 60 IU/mL. The detection rates at 20 IU/mL and 6 IU/mL were 93% and 61%, respectively (Table 21). Detection rates were calculated from valid initial results. Due to the cross reactivity of this standard with HBV,[34] only the dHIV-1 Assay was tested.

Table 21. PROCLEIX® System - Detection of HIV-1 WHO Standard in Analytical Sensitivity Panels with the PROCLEIX® HIV-1 Discriminatory Assay

HIV-1 WHO (97/656) IU/mL	Number of reactive/ tested*	% Positive	95% Confidence Limits		Average S/CO	%CV
			Lower	Upper		
600	119/119	100	97	100	23.48	13
200	120/120	100	97	100	22.58	12
60	119/119	100	97	100	20.56	17
20	110/118	93	87	97	14.28	43
6	73/120	61	52	70	11.17	57
0	0/120	0	0	3	0.10	59

***** Invalid reactions were not included.

PROCLEIX® System - Detection of HCV WHO Standard (96/790)

The detection rate for the HCV WHO standard at 100 and 30 IU/mL was 100% and ≥99% at 10 IU/mL for both the PROCLEIX® ULTRIO® Assay and HCV Discriminatory Assay (dHCV). The detection rate at 3 IU/mL in the PROCLEIX ULTRIO Assay was 91%. In the dHCV Assay, the detection rate at 3 IU/mL was 96%. The detection rates for 1 IU/mL were 64% and 67% for the PROCLEIX ULTRIO Assay and dHCV Assay. There were no statistically significant differences observed in the positivity rates for the detection of HCV WHO standard with the PROCLEIX ULTRIO Assay and dHCV Assay (Table 22). Detection rates were calculated from valid initial results.

Table 22. PROCLEIX® System - Detection of HCV WHO Standard in Analytical Sensitivity Panels

	PROCLEIX® ULTRIO® Assay						dHCV					
HCV WHO (96/790) IU/mL	Number of reactive/ tested*	% Positive	95% Confidence Limits		Average S/CO	%CV	Number of reactive/ tested*	% Positive	95% Confidence Limits		Average S/CO	%CV
			Lower	Upper					Lower	Upper		
100	118/118	100	97	100	7.45	5	120/120	100	97	100	21.97	9
30	119/119	100	97	100	7.32	5	118/118	100	97	100	21.51	8
10	119/120	99	95	100	7.10	8	120/120	100	97	100	20.84	12
3	109/120	91	84	95	6.52	19	115/120	96	91	99	18.88	21
1	77/120	64	55	73	5.80	28	79/118	67	58	75	17.40	32
0	0/120	0	0	3	0.09	52	0/120	0	0	3	0.11	104

***** Invalid reactions were not included.

PROCLEIX® System - Detection of HBV WHO Standard (97/746)

The detection rate of the PROCLEIX® ULTRIO® Assay and the HBV Discriminatory Assay (dHBV) was 100% for HBV WHO standard at 45 IU/mL and ≥99% at 15 IU/mL. HBV detection at 5 IU/mL with the PROCLEIX ULTRIO Assay and the dHBV Assay was 74% and 77% respectively, at 1.67 IU/mL detection rates were 40% and 41% respectively and 19% and 18% respectively for 0.56 IU/mL. There were no statistically significant differences observed in the positivity rates for the detection of HBV WHO standard with the PROCLEIX ULTRIO Assay and dHBV Assay (Table 23). Detection rates were calculated from valid initial results.

Table 23. PROCLEIX® System - Detection of HBV WHO Standard in Analytical Sensitivity Panels

PROCLEIX® ULTRIO® Assay							dHBV					
HBV WHO (97/746) IU/mL	Number of reactive/ tested*	% Positive	95% Confidence Limits		Average S/CO	%CV	Number of reactive/ tested*	% Positive	95% Confidence Limits		Average S/CO	%CV
			Lower	Upper					Lower	Upper		
45	120/120	100	97	100	14.27	7	119/119	100	97	100	22.70	9
15	119/120	99	95	100	13.91	12	120/120	100	97	100	22.05	13
5	89/120	74	65	82	11.18	36	91/119	77	68	84	17.93	38
1.67	48/120	40	31	49	11.89	32	49/119	41	32	51	17.75	38
0.56	22/119	19	12	27	9.95	48	21/120	18	11	26	16.15	51
0	0/119	0	0	3	0.12	73	0/120	0	0	3	0.07	129

***** Invalid reactions were not included.

PROCLEIX® System - Probit Analysis

The predicted 50% and 95% detection rates in copies/mL or IU/mL for each target were determined with Probit Analysis of the analytical sensitivity results. The predicted 95% detection rate for HIV-1 type B was 37.7 copies/mL for the PROCLEIX® ULTRIO® Assay and 35.4 copies/mL for the dHIV-1 Assay. The predicted 95% detection rate for HIV-1 WHO was 18.1 IU/mL for the dHIV-1 Assay. The predicted 95% detection rate for HCV WHO was 3.7 IU/mL and 2.4 IU/mL for the PROCLEIX ULTRIO Assay and the dHCV Assay, respectively. The 95% detection rate for HBV was 8.0 IU/mL and 6.8 IU/mL for the PROCLEIX ULTRIO Assay and dHBV Assay, respectively (Table 24).

Table 24. PROCLEIX® System - Detection Probabilities of HIV-1, HCV, and HBV

Panel Tested	Assay	Detection Probabilities	
		50% (95% Fiducial Limits)	95% (95% Fiducial Limits)
HIV-1 B copies/mL	PROCLEIX® ULTRIO® Assay	13.9 (12.0-15.9)	37.7 (33.6-43.0)
HIV-1 B copies/mL	dHIV-1	12.9 (11.2-14.6)	35.4 (33.8-36.9)
HIV-1 WHO (97/656) IU/mL	dHIV-1	7.5 (6.4-8.7)	18.1 (16.1-20.8)
HCV WHO (96/790) IU/mL	PROCLEIX ULTRIO Assay	1.3 (1.0-1.5)	3.7 (3.3-4.2)
HCV WHO (96/790) IU/mL	dHCV	1.0 (0.9-1.2)	2.4 (2.1-2.7)
HBV WHO (97/746) IU/mL	PROCLEIX ULTRIO Assay	3.3 (3.0-3.8)	8.0 (7.1-9.3)
HBV WHO (97/746) IU/mL	dHBV	3.0 (2.7-3.4)	6.8 (6.0-7.7)

SENSITIVITY OF DETECTION FOR HIV-1, HCV, AND HBV GENETIC VARIANTS

Multiple specimens and tissue culture isolates were tested to determine the sensitivity of detection of the viral genetic variants.

PROCLEIX® System - Detection of HIV-1 Genetic Variants with the PROCLEIX® ULTRIO® Assay and HIV-1 Discriminatory Assay

HIV-1 specimens and tissue culture isolates of group M (subtypes A, B, C, D, E, F, and G), N, and O were quantified for HIV-1 RNA concentrations using commercially available quantitative HIV-1 RNA assays or with an in-house quantitative HIV-1 RNA test. Specimens were diluted with negative human plasma to target viral concentrations of 300, 100 and 30 copies/mL. Diluted specimens were tested in the PROCLEIX® ULTRIO® Assay and dHIV-1 Assay. Fifty-four unique specimens or tissue culture isolates were tested in duplicate using three clinical lots on the PROCLEIX® System. Six of the specimens were co-infected with HCV and/or HBV and were therefore only tested in the dHIV-1 Assay. At 300 copies/mL, 287/288 replicates (99.7%) were reactive with the PROCLEIX ULTRIO Assay and 324/324 replicates (100%) were reactive with the dHIV-1 Assay. At 100 copies/mL, 286/288 replicates (99.3%) were reactive with the PROCLEIX ULTRIO Assay and 320/324 replicates (98.8%) were reactive with the dHIV-1 Assay. At 30 copies/mL, 252/288 replicates (87.5%) were reactive with the PROCLEIX ULTRIO Assay and 289/324 replicates (89.2%) were reactive with the dHIV-1 Assay. (Table 25). Detection rates were calculated from valid initial results.

Table 25. PROCLEIX® System - Detection of HIV-1 Genetic Variants with the PROCLEIX® ULTRIO® Assay and HIV-1 Discriminatory Assay

Genetic Variant	Conc. copies/mL	PROCLEIX® ULTRIO® Assay			HIV-1 Discriminatory Assay		
		Unique Donors*	Reactive/ Tested	% Reactive	Unique Donors*	Reactive/ Tested	% Reactive
HIV-1 Group M Subtype A	300	8	48/48	100	9	54/54	100
	100		48/48	100		54/54	100
	30		46/48	95.8		48/54	88.9
HIV-1 Group M Subtype B	300	6	36/36	100	7	42/42	100
	100		36/36	100		42/42	100
	30		30/36	83.3		40/42	95.2
HIV-1 Group M Subtype C	300	8	48/48	100	8	48/48	100
	100		48/48	100		47/48	97.9
	30		42/48	87.5		42/48	87.5
HIV-1 Group M Subtype D	300	6	36/36	100	6	36/36	100
	100		36/36	100		36/36	100
	30		34/36	94.4		32/36	88.9
HIV-1 Group M Subtype E	300	8	48/48	100	9	54/54	100
	100		48/48	100		54/54	100
	30		45/48	93.8		51/54	94.4
HIV-1 Group M Subtype F	300	3	18/18	100	5	30/30	100
	100		18/18	100		30/30	100
	30		13/18	72.2		25/30	83.3
HIV-1 Group M Subtype G	300	1	6/6	100	2	12/12	100
	100		6/6	100		12/12	100
	30		6/6	100		12/12	100
HIV-1 Group N	300	1	5/6	83.3	1	6/6	100
	100		4/6	66.7		3/6	50
	30		3/6	50		2/6	33.3
HIV-1 Group O	300	7	42/42	100	7	42/42	100
	100		42/42	100		42/42	100
	30		33/42	78.6		37/42	88.1
All Genotypes	300	48	287/288	99.7	54	324/324	100
	100		286/288	99.3		320/324	98.8
	30		252/288	87.5		289/324	89.2

* Each unique donor was tested in duplicate with three clinical lots of reagents.

PROCLEIX® System - Detection of HCV Genotypes with the PROCLEIX® ULTRIO® Assay and HCV Discriminatory Assay

HCV specimens of genotypes 1, 2, 3, 4, 5, and 6 were quantified for HCV RNA using commercially available quantitative HCV RNA assays. Specimens were diluted with negative human plasma to target viral concentrations of 300, 100 and 30 copies/mL. The diluted specimens were tested with the PROCLEIX® ULTRIO® Assay and dHCV Assay. Sixty-one unique specimens were tested in duplicate using three clinical lots on the PROCLEIX® System. One specimen was co-infected with HIV-1 and was therefore only tested in the dHCV Assay. At 300 copies/mL, all replicates were reactive with both the PROCLEIX ULTRIO Assay and the dHCV Assay. At 100 copies/mL, 354/360 replicates (98.3%) were reactive with the PROCLEIX ULTRIO Assay and 357/366 replicates (97.5%) were reactive with the dHCV Assay. At 30 copies/mL, 330/360 replicates (91.7%) were reactive with the PROCLEIX ULTRIO Assay and 337/366 replicates (92.1%) were reactive with the dHCV Assay. (Table 26). Detection rates were calculated from valid initial results.

Table 26. PROCLEIX® System - Detection of HCV Genotypes with the PROCLEIX® ULTRIO® Assay and HCV Discriminatory Assay

Genotype	Conc. copies/mL	PROCLEIX® ULTRIO® Assay			HCV Discriminatory Assay		
		Unique Donors*	Reactive/ Tested	% Reactive	Unique Donors*	Reactive/ Tested	% Reactive
HCV Genotype 1	300	11	66/66	100	11	66/66	100
	100		66/66	100		66/66	100
	30		59/66	89.4		62/66	93.9
HCV Genotype 2	300	12	72/72	100	13	78/78	100
	100		67/72	93.1		73/78	93.6
	30		62/72	86.1		67/78	85.9
HCV Genotype 3	300	12	72/72	100	12	72/72	100
	100		71/72	98.6		69/72	95.8
	30		65/72	90.3		64/72	88.9
HCV Genotype 4	300	14	84/84	100	14	84/84	100
	100		84/84	100		83/84	98.8
	30		81/84	96.4		80/84	95.2
HCV Genotype 5	300	6	36/36	100	6	36/36	100
	100		36/36	100		36/36	100
	30		35/36	97.2		35/36	97.2
HCV Genotype 6	300	5	30/30	100	5	30/30	100
	100		30/30	100		30/30	100
	30		28/30	93.3		29/30	96.7
All Genotypes	300	60	360/360	100	61	366/366	100
	100		354/360	98.3		357/366	97.5
	30		330/360	91.7		337/366	92.1

* Each unique donor was tested in duplicate with three clinical lots of reagents.

PROCLEIX® System - Detection of HBV Genotypes with the PROCLEIX® ULTRIO® Assay and HBV Discriminatory Assay

HBV specimens of genotypes A, B, C, D, E, F, and G were quantified for HBV DNA using commercially available quantitative HBV DNA assays. Specimens were diluted with negative human plasma to target viral concentrations of 300, 100 and 30 copies/mL. Diluted specimens were tested with the PROCLEIX® ULTRIO® Assay and dHBV Assay. Fifty-seven unique specimens were tested in duplicate using three clinical lots on the PROCLEIX® System. At 300 copies/mL, 337/342 replicates (98.5%) were reactive with the PROCLEIX ULTRIO Assay and 337/342 replicates (98.5%) were reactive with the dHBV Assay. At 100 copies/mL, 324/342 replicates (94.7%) were reactive with the PROCLEIX ULTRIO Assay and 312/342 replicates (91.2%) were reactive with the dHBV Assay. At 30 copies/mL, 265/342 replicates (77.5%) were reactive with the PROCLEIX ULTRIO Assay and 244/342 replicates (71.3%) were reactive with the dHBV Assay. (Table 27). Detection rates were calculated from valid initial results.

Table 27. PROCLEIX® System - Detection of HBV Genotype with the PROCLEIX® ULTRIO® Assay and HBV Discriminatory Assay

Genotype	Conc. copies/mL	PROCLEIX® ULTRIO® Assay			HBV Discriminatory Assay		
		Unique Donors*	Reactive/ Tested	% Reactive	Unique Donors*	Reactive/ Tested	% Reactive
HBV Genotype A	300	12	71/72	98.6	12	70/72	97.2
	100		70/72	97.2		67/72	93.1
	30		63/72	87.5		57/72	79.2
HBV Genotype B	300	10	60/60	100	10	60/60	100
	100		57/60	95		56/60	93.3
	30		43/60	71.7		36/60	60
HBV Genotype C	300	10	60/60	100	10	59/60	98.3
	100		52/60	86.7		54/60	90
	30		41/60	68.3		41/60	68.3
HBV Genotype D	300	8	45/48	93.8	8	47/48	97.9
	100		46/48	95.8		44/48	91.7
	30		41/48	85.4		35/48	72.9
HBV Genotype E	300	8	47/48	97.9	8	48/48	100
	100		46/48	95.8		40/48	83.3
	30		32/48	66.7		34/48	70.8
HBV Genotype F	300	8	48/48	100	8	47/48	97.9
	100		47/48	97.9		45/48	93.8
	30		39/48	81.3		35/48	72.9
HBV Genotype G	300	1	6/6	100	1	6/6	100
	100		6/6	100		6/6	100
	30		6/6	100		6/6	100
All Genotypes	300	57	337/342	98.5	57	337/342	98.5
	100		324/342	94.7		312/342	91.2
	30		265/342	77.5		244/342	71.3

* Each unique donor was tested in duplicate with three clinical lots of reagents.

PERFORMANCE OF THE PROCLEIX ULTRIO ASSAY IN CADAVERIC BLOOD SPECIMENS FROM TISSUE DONORS

Note: All performance evaluations were performed on cadaveric serum specimens, unless otherwise noted.

REPRODUCIBILITY

The reproducibility of the PROCLEIX® ULTRIO® Assay and Discriminatory Assays with cadaveric blood specimens was assessed on the PROCLEIX® System. Plasma containing HIV-1, HCV or HBV was spiked into 20 cadaveric and 20 control specimens (HIV-1 at 200 copies/mL, HCV at 60 IU/mL and HBV at 45 IU/mL); 10 of each were tested in the PROCLEIX ULTRIO Assay and the other 10 of each were tested in the Discriminatory Assays. The specimens were tested with two clinical lots. Specimens were tested in three separate runs for each clinical lot, for a total of six runs. The percent positive, analyte S/CO values, and coefficients of variation (%CVs) are shown in Table 28. Detection rates were calculated from valid initial results. The positivity rates ranged from 98% to 100% on the PROCLEIX System. The %CVs ranged from 28% to 32% for HIV-1 spiked cadaveric specimens, 29% to 36% for the HCV spiked cadaveric specimens, and 7% to 8% for the HBV spiked cadaveric specimens.

Table 28. PROCLEIX® System - Reproducibility of PROCLEIX® ULTRIO® Assay and Discriminatory Assays in Cadaveric Blood Specimens

Virus	Assay	Sample Type*	# of Donors	# of Replicates	% Positive	Mean Analyte S/CO	%CV
HIV-1	PROCLEIX® ULTRIO® Assay	Cadaveric	10	60	100% (95-100)	14.80	28
		Control	10	60	98% (91-100)	12.05	21
	dHIV-1	Cadaveric	10	57**	98% (91-100)	15.04	32
		Control	10	60	100% (95-100)	18.89	17
HCV	PROCLEIX® ULTRIO® Assay	Cadaveric	10	60	98% (91-100)	5.93	36
		Control	10	60	100% (95-100)	6.83	8
	dHCV	Cadaveric	10	60	98% (91-100)	16.17	29
		Control	10	60	100% (95-100)	21.96	13
HBV	PROCLEIX® ULTRIO® Assay	Cadaveric	10	60	98% (91-100)	13.55	8
		Control	10	60	98% (91-100)	13.51	7
	dHBV	Cadaveric	10	60	100% (95-100)	23.05	7
		Control	10	60	100% (95-100)	23.74	5

CI = Confidence Interval
* Cadaveric specimens included serum and plasma specimens.
** Three specimens with invalid IC, QNS for retest.

SPECIFICITY

Specificity of PROCLEIX® ULTRIO® Assay and Discriminatory Assays in Cadaveric Blood Specimens on the PROCLEIX® System

HIV-1, HCV and HBV seronegative cadaveric blood specimens were tested to determine the specificity of the PROCLEIX® ULTRIO® Assay and dHIV-1, dHCV and dHBV Assays. Approximately 50 cadaveric and 50 normal donor specimens were tested using three clinical lots on the PROCLEIX® System. The specificity of the PROCLEIX ULTRIO Assay and dHIV-1 and dHBV Assays for the cadaveric specimens was 100% (95% confidence interval: 94%-100%) for the PROCLEIX System. The specificity of the dHCV Assay for the cadaveric specimens was 98% (95% confidence interval: 89%-100%) (Table 29). Specificity rates were calculated from all valid initial results.

Table 29. PROCLEIX® System - Specificity of PROCLEIX® ULTRIO® Assay and Discriminatory Assays in Cadaveric Blood Specimens

		Control	Cadaveric
PROCLEIX® ULTRIO® Assay	Mean IC S/CO	1.94	1.89
	Mean Analyte S/CO	0.05	0.09
	Specificity rate	100 %	100 %
	95% CI spec. rate	94-100	94-100
	N	50	47
dHIV-1	Mean IC S/CO	2.00	1.99
	Mean Analyte S/CO	0.14	0.11
	Specificity rate	100 %	100 %
	95% CI spec. rate	94-100	94-100
	N	50	50
dHCV	Mean IC S/CO	2.07	1.98
	Mean Analyte S/CO	0.14	0.21
	Specificity rate	100 %	98* %
	95% CI spec. rate	94-100	89-100
	N	50	49
dHBV	Mean IC S/CO	2.01	2.02
	Mean Analyte S/CO	0.11	0.10
	Specificity rate	100 %	100 %
	95% CI spec. rate	94-100	94-100
	N	50	49

* One initial reactive, QNS to resolve
CI = Confidence Interval
N = number of samples

SENSITIVITY

Sensitivity for Detection of HIV-1 in Cadaveric Blood Specimens on the PROCLEIX® System

HIV-1, HCV, and HBV seronegative cadaveric blood specimens spiked with a low level of HIV-1 virus were tested to determine the sensitivity of the PROCLEIX® ULTRIO® Assay and dHIV-1 Assay. Approximately 50 cadaveric and 50 normal donor specimens were tested using three clinical lots on the PROCLEIX® System after spiking each with approximately 200 copies/mL of HIV-1. The positivity rate of the PROCLEIX ULTRIO Assay and dHIV-1 Assay for the cadaveric specimens was 100% (95% confidence interval: 94%-100%) on the PROCLEIX System (Table 30). Detection rates were calculated from valid initial results.

Table 30. PROCLEIX® System - Reactivity of PROCLEIX® ULTRIO® Assay and HIV-1 Discriminatory Assay in Cadaveric Blood Specimens Spiked with HIV-1 Virus

		Control	Cadaveric
PROCLEIX® ULTRIO® Assay	Mean IC S/CO	2.36	2.31
	Mean Analyte S/CO	12.85	12.05
	% positive	100	100
	95% CI (% pos)	94-100	94-100
	N	50	50
dHIV-1	Mean IC S/CO	2.12	2.07
	Mean Analyte S/CO	17.01	15.89
	% positive	100	100
	95% CI (% pos)	94-100	94-100
	N	50	50

CI = Confidence Interval
N = number of samples

Sensitivity for Detection of HCV in Cadaveric Blood Specimens on the PROCLEIX® System

HIV-1, HCV and HBV seronegative cadaveric blood specimens spiked with a low level of HCV virus were tested to determine the sensitivity of the PROCLEIX® ULTRIO® Assay and dHCV Assay. Approximately 50 cadaveric and 50 normal donor specimens were tested using three clinical lots on the PROCLEIX® System after spiking each with approximately 200 copies/mL of HCV. The positivity rate of both the PROCLEIX ULTRIO Assay and dHCV Assay for the cadaveric specimens was 100% (95% confidence interval: 94%-100%) on the PROCLEIX System (Table 31). Detection rates were calculated from valid initial results.

Table 31. PROCLEIX® System - Reactivity of PROCLEIX® ULTRIO® Assay and HCV Discriminatory Assay in Cadaveric Blood Specimens Spiked with HCV Virus

		Control	Cadaveric
PROCLEIX® ULTRIO® Assay	Mean IC S/CO	2.03	1.95
	Mean Analyte S/CO	7.93	7.05
	% positive	100	100
	95% CI (% pos)	94-100	94-100
	N	50	50
dHCV	Mean IC S/CO	2.03	1.91
	Mean Analyte S/CO	22.06	19.35
	% positive	100	100
	95% CI (% pos)	94-100	94-100
	N	50	50

CI = Confidence Interval
N = number of samples

Sensitivity for Detection of HBV in Cadaveric Blood Specimens on the PROCLEIX® System

HIV-1, HCV and HBV seronegative cadaveric blood specimens spiked with a low level of HBV virus were tested to determine the sensitivity of the PROCLEIX® ULTRIO® Assay and dHBV Assay. Seventy cadaveric and 70 normal donor specimens were tested using three clinical lots on the PROCLEIX®System after spiking each with approximately 30 IU/mL of HBV. The positivity rate of the PROCLEIX ULTRIO Assay for the cadaveric specimens was 100% (95% confidence interval: 96%-100%) on the PROCLEIX System. The positivity rate of the dHBV Assay was 98% (95% confidence interval: 92%-100%) for the PROCLEIX System (Table 32). Detection rates were calculated from valid initial results.

Table 32. PROCLEIX® System - Reactivity of PROCLEIX® ULTRIO® Assay and HBV Discriminatory Assay in Cadaveric Blood Specimens Spiked with HBV Virus

		Control	Cadaveric*
PROCLEIX® ULTRIO® Assay	Mean IC S/CO	1.66	1.56
	Mean Analyte S/CO	13.39	12.92
	% positive	100	100
	95% CI (% pos)	96-100	96-100
	N	70	70
dHBV	Mean IC S/CO	1.89	1.72
	Mean Analyte S/CO	21.75	21.54
	% positive	100	98
	95% CI (% pos)	96-100	92-100
	N	70	70

CI = Confidence Interval
N = number of samples
* Included serum and plasma specimens

REACTIVITY IN SEROCONVERTING DONORS

PROCLEIX® SYSTEM

Commercially available seroconversion panels collected from plasmapheresis donors were tested to determine the ability of the PROCLEIX® ULTRIO® Assay and HIV-1, HCV, and HBV Discriminatory Assays to shorten the window period of HIV-1, HCV, and HBV detection when compared to antigen and/or antibody tests. Two separate studies were performed, each with a different clinical lot (Tables 33a and 33b). Both studies tested a similar set of HIV-1 (n=10), HCV (n=10), and HBV (n=10) seroconversion panels at one site. Each seroconversion panel was tested with the PROCLEIX ULTRIO Assay (either neat and 1:16 diluted in one study or neat and 1:8 diluted in the other study which used development clinical lots) and with the HIV-1 Discriminatory (neat only), HCV Discriminatory (neat only) and HBV Discriminatory (neat only) Assays. The test results were compared with those of the Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA Assay and the Coulter HIV-1 p24 Ag Assay for HIV-1 seroconversion panels, with those of the Ortho HCV 3.0 ELISA test for HCV seroconversion panels, or with those of Ortho Antibody to HBsAg ELISA Test System 3 and Abbott PRISM HBsAg Assay for HBV seroconversion panels.

HIV-1 Detection in Seroconversion Panels

The PROCLEIX® ULTRIO® Assay was able to detect HIV-1 RNA a median of 14 and 7 (or 14 and 8 in the second study) days earlier than the Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA Assay and the Coulter HIV-1 p24 Ag Assay, respectively, when specimens were tested neat (Tables 33a and 33b). The PROCLEIX ULTRIO Assay was able to detect HIV-1 RNA a median of 11.5 and 4 days earlier than the Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA Assay and the Coulter HIV-1 p24 Ag Assay, respectively, when specimens were tested at a 1:8 dilution. The PROCLEIX ULTRIO Assay was able to detect HIV-1 RNA a median of 11 and 5 days earlier than the Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA Assay and the Coulter HIV-1 p24 Ag Assay, respectively, when specimens were tested at a 1:16 dilution. Similar results were observed with the HIV-1 Discriminatory Assay, as compared to the PROCLEIX ULTRIO Assay, when specimens were tested neat in both studies.

Table 33a. PROCLEIX® System - Comparison to HIV-1 Antibody and Antigen Tests on Seroconversion Panels Study 1 (Number of Days Earlier Detection)

Panel ID	Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA Assay			Coulter HIV-1 p24 Ag Assay		
	PROCLEIX® ULTRIO® Assay (Neat)	PROCLEIX® ULTRIO® Assay (Diluted 1:16)	dHIV-1 (Neat)	PROCLEIX® ULTRIO® Assay (Neat)	PROCLEIX® ULTRIO® Assay (Diluted 1:16)	dHIV-1 (Neat)
60772	12	7	12	7	2	7
61694	11	8	8	6	3	3
62238	14	20	14	7	13	7
62357	11	7	11	4	0	4
65389	14	12	14	7	5	7
65790*	>11	>7	>11	7	3	7
66048	15	12	15	22	19	22
67485	18	14	18	8	4	8
68106*	14	10	14	>46	>42	>46
68582	14	14	14	7	7	7
Median	14	11	14	7	5	7

* Panel did not show Ab or Ag reactivity.

Table 33b. PROCLEIX® System - Comparison to HIV-1 Antibody and Antigen Tests on Seroconversion Panels Study 2 (Number of Days Earlier Detection)

Panel ID	Abbott HIVAB HIV-1/HIV-2 (rDNA) EIA Assay			Coulter HIV-1 p24 Ag Assay		
	PROCLEIX® ULTRIO® Assay (Neat)	PROCLEIX® ULTRIO® Assay (Diluted 1:8)	dHIV-1 (Neat)	PROCLEIX® ULTRIO® Assay (Neat)	PROCLEIX® ULTRIO® Assay (Diluted 1:8)	dHIV-1 (Neat)
60772	12	7	7	7	2	2
62357*	11	7	11	4	0	4
63602	16	9	14	9	2	7
64954*	15	13	15	15	13	15
65790*	12	7	12	8	3	8
66575	14	11	11	10	7	7
67485*	14	14	14	4	4	4
68582	14	14	14	7	7	7
66048*	15	12	15	22	19	22
68106*,**	15	15	19	>54	>54	>54
Median	14	11.5	14	8	4	7

* Intermittent PROCLEIX ULTRIO Assay reactivity prior to ramp up is not used for this calculation
** Panel did not show Ag reactivity.

HCV Detection in Seroconversion Panels

In both studies the PROCLEIX® ULTRIO® Assay was able to detect HCV RNA a median of 32 days earlier than the Ortho HCV 3.0 ELISA test when specimens were tested neat, at 1:8 dilution, and at 1:16 dilution (Tables 34a and 34b). The HCV Discriminatory Assay was able to detect HCV RNA a median of 32 days and 34.5 days earlier than the Ortho HCV 3.0 ELISA Assay when specimens were tested neat in the two separate studies.

Table 34a. PROCLEIX® System - Comparison to Ortho HCV 3.0 ELISA Assay on Seroconversion Panels
Study 1 (Number of Days Earlier Detection)

Panel ID	PROCLEIX® ULTRIO® Assay (Neat)	PROCLEIX® ULTRIO® Assay (Diluted 1:16)	dHCV (Neat)
60779	0	Not Reactive*	0
61067	32	32	32
62286	23	23	23
62680**	>22	>22	>22
62804	20	20	20
62886	31	31	31
62999	39	33	64
63318	32	32	32
63625	62	38	38
790989	46	46	46
Median	32	32	32

* Panel did not have a reactive NAT result
** Panel did not show Ab reactivity

Table 34b. PROCLEIX® System - Comparison to Ortho HCV 3.0 ELISA Assay on Seroconversion Panels
Study 2 (Number of Days Earlier Detection)

Panel ID	PROCLEIX® ULTRIO® Assay (Neat)	PROCLEIX® ULTRIO® Assay (Diluted 1:8)	dHCV (Neat)
790989	44	44	44
61067	27	27	30
60779	0	0	0
62286	23	23	23
62999	33	33	39
63318	52	34	52
62886*	31	31	31
63625	38	38	38
64150*	46	46	46
64273*	29	29	29
Median	32	32	34.5

* Intermittent PROCLEIX ULTRIO Assay reactivity prior to ramp up is not used for this calculation.

HBV Detection in Seroconversion Panels

The PROCLEIX® ULTRIO® Assay was able to detect HBV DNA a median of 19 days and 17 (or 18.5 in the second study) days earlier than the Ortho Antibody to HBsAg ELISA Test System 3 and the Abbott PRISM HBsAg Assay, respectively, when specimens were tested neat (Tables 35a and 35b). The PROCLEIX ULTRIO Assay was able to detect HBV DNA a median of 11.5 days earlier than the Abbott PRISM HBsAg Assay when specimens were tested at 1:8 dilution. The PROCLEIX ULTRIO Assay was able to detect HBV DNA a median of 9 days and 7 days earlier than the Ortho Antibody to HBsAg ELISA Test System 3 and the Abbott PRISM HBsAg Assay, respectively, when specimens were tested at 1:16 dilution. The HBV Discriminatory Assay was able to detect HBV DNA a median of 16 days and 15 (or 17 in the second study) days earlier than the Ortho Antibody to HBsAg ELISA Test System 3 and the Abbott PRISM HBsAg Assay, respectively, when specimens were tested neat.

Table 35a. PROCLEIX® System - Comparison to HBV Surface Antigen Tests on Seroconversion Panels (Number of Days Earlier Detection)

Panel ID	Ortho Antibody to HBsAg ELISA Test System 3			Abbott PRISM HBsAg Assay		
	PROCLEIX® ULTRIO® Assay (Neat)	PROCLEIX® ULTRIO® Assay (Diluted 1:16)	dHBV (Neat)	PROCLEIX® ULTRIO® Assay (Neat)	PROCLEIX® ULTRIO® Assay (Diluted 1:16)	dHBV (Neat)
62675	19	17	19	19	17	19
62825	29	29	29	29	29	29
62967	14	5	14	14	5	14
63133	11	9	11	11	9	11
63568	14	11	14	10	7	10
63659	15	0	0	15	0	0
63997	21	7	14	19	5	12
64006	20	8	18	20	8	18
64121	19	0	27	19	0	27
64132	23	14	23	15	6	15
Median	19	9	16	17	7	15

Table 35b. PROCLEIX® System - Comparison to Abbott PRISM HBsAg Assay on Seroconversion Panels (Number of Days Earlier Detection)

Panel ID	PROCLEIX® ULTRIO® Assay (Neat)	PROCLEIX® ULTRIO® Assay (Diluted 1:8)	dHBV (Neat)
62825	17	3	17
62347	11	7	11
62967*	10	3	12
64121*	19	19	17
64006*	23	11	23
66201*	21	21	23
67303*	22	12	19
68029	18	16	16
68105	29	29	29
68739*	15	6	15
Median	18.5	11.5	17

* Intermittent PROCLEIX ULTRIO Assay reactivity prior to ramp up is not used for this calculation.

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